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SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Fifty-fifth meeting.

Cornell University Medical College, October 15, 1913.

President Ewing in the chair.

1 (818)

On reversible emulsions and the role played by electrolytes in determining the equilibrium of aqueous oil systems.

By G. H. A. Clowes.

[*From the Biological Chemical Department of the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

The "oil-water" emulsion obtained by shaking equal volumes of olive oil and water with a sufficient amount of $n/10$ NaOH to render the external aqueous phase alkaline to phenol phthalein, may be readily converted into a "water-oil" emulsion by shaking with a solution containing an amount of $CaCl_2$ slightly in excess of the chemical equivalent of the NaOH employed in the preparation of the "oil-water" emulsion. The resulting "water-oil" emulsion may be re-converted into an "oil-water" emulsion by shaking with further additions of NaOH until the total amount of that substance in the system slightly exceeds the chemical equivalent of the $CaCl_2$ previously employed.

When chemically equivalent proportions of Ca and OH are employed in a system containing equal volumes of oil and the aqueous phase, neither type of emulsion appears to predominate. At this critical point the system is in a state of extremely unstable equilibrium. Shaking with a trace of NaOH solution converts it almost instantaneously into an "oil-water" emulsion, while a trace of $CaCl_2$ exerts the reverse effect, a "water-oil" emulsion being formed.

Magnesium functions in a manner similar to calcium, one ion being equivalent to two of OH; iron and aluminium exert a greater effect, one ion being equivalent to three of OH, at the critical point, provided equal volumes of oil and water have been employed. NaCl exerts no effect on a system of this type. If certain oil-water systems are slowly transformed through the critical point to water-oil systems or vice versa, figures resembling those in karyokinesis may be observed microscopically.

Bancroft[1] concludes that the production of a stable emulsion depends on the formation of a concentration film or membrane at all points of contact between the dispersed and continuous phases, and that the relative solubility of this film in the aqueous and oil phases and the consequent surface tension relations on its two sides determine the nature of the emulsion formed. From the above experiments it appears probable that positive ions adsorbed by a stabilizing fatty acid film render the later relatively more soluble in the oil or less soluble in the water phase thus lowering the surface tension on the oil as compared with the water side. The tension on its two faces being unequal, the membrane tends to develop a spherical curvature, the concave side being presented to the water and the convex side to the oil phase. This causes the production of a "water-oil" emulsion, the number and size of the water globules formed depending on the relative proportion of positive ions adsorbed by the film and the consequent differences in surface tension on its two sides. The adsorption of negative ions on the other hand renders the membrane more soluble in the aqueous phase, and consequently lowers the surface tension on that side causing the membrane to curve in such a manner as to present its concave face to the oil phase, an "oil-water" emulsion being formed. At the critical point the proportion of positive and negative ions adsorbed by the membrane must be such that the surface tension remains practically the same on both sides of the film which consequently fails to develop a curvature in either direction. Further support is lent to this point of view by the fact that the fatty acid salts of calcium, magnesium, iron, aluminium, etc., are relatively freely soluble in oil and relatively insoluble in water. In order to deter-

[1] *Journal of Physical Chemistry*, 1913, 17, p. 501.

mine more accurately the relative capacity possessed by different positive ions to inhibit the solvent effect of negative ions on the stabilizing fatty acid film formed by contact between aqueous and oil phases, solutions containing varying proportions of positive and negative ions were run through oil from a Traube stalagmometer. By observing the relative number of drops formed by equal volumes of different solutions, or the concentration of solutions giving an equal number of drops, it was possible to determine the relative facility with which films of this nature were formed and maintained. It was found that equal volumes of $m/1000$ NaOH, $m/50$ $Ca(OH)_2$ and $m/10$ $Fe(OH)_3$ gave an equal number of drops. Furthermore the concentrations of NaCl, $CaCl_2$ and $FeCl_3$ required to precipitate a sodium oleate solution were found to be .4 *M*, .02 *M*, and .004 *M*, respectively. Since these ratios of 100 : 5 : 1 are the inverse of those observed above, and since they represent approximately the logarithmic ratio observed by Hardy for the precipitation of negatively charged colloids by mono-, di-, and trivalent kations, it may be concluded that the maintenance of equilibrium of an aqueous oil system of this type depends upon the relative proportion of kations and anions present in the system and adsorbed by the stabilizing film and the extent to which the adsorbed kations, by counteracting the solvent action of adsorbed anions promote the continuity of the stabilizing film. From the experiments of Loeb, and his pupils, it must be concluded that di- and trivalent kations exert a far greater effect than monovalent kations in maintaining the equilibrium of biological systems. Since these ratios are frequently approximately logarithmic, the question suggests itself whether the positive ions concerned do not function by counteracting the destructive effect of negative ions on protoplasmic films or membranes in a manner similar to that indicated above for purely physical systems.

2 (819)

On analogous effects exerted by antagonistic calcium and citrate ions in physical and biological systems.

By G. H. A. CLOWES.

[*From the Biological Chemical Department of the State Institute for the Study of Malignant Diseases, Buffalo, N. Y.*]

From the preceding paper it appears that antagonistic kations and anions exert their effect upon the equilibrium of aqueous oil systems by causing variations in the relative solubility of a concentration film of fatty acid salts in oil and water. The resemblance between the effects observed in purely physical systems of this type and in biological systems suggests the possibility that protoplasm may consist essentially of an aqueous lipoid system in which a film of lipoid material functions as the continuous phase. If this is the case, an excess of either positive or negative ions should exert a disturbing effect upon the physical equilibrium of the protoplasm corresponding with that observed above, and a critical point at which kations and anions exactly counterbalance one another should be found in biological systems corresponding with that observed in purely physical systems, the precipitation of lipoids and fatty acid salts, for example.

To test this question calcium was selected as a suitable kation, and citrate as a suitable anion on account of the facility with which the proportions in which they counterbalance one another in the process of blood coagulation may be estimated (see subsequent communication). A $m/5$ $CaCl_2$ and a chemically equivalent solution of sodium citrate were prepared and admixed in varying proportions ranging from 0 per cent. of Ca and 100 per cent. of citrate to 100 per cent. of Ca and 0 per cent. of citrate. On mixing these solutions with aqueous sodium oleate suspension, the following results were obtained.

Precipitation was complete from 100 per cent. to 40 per cent. of calcium, gradually diminishing from this point until virtually no precipitation was visible between 35 per cent. and 30 per cent. of calcium, once more rising to an almost complete precipitation between 25 per cent. and 5 per cent. and subsequently diminishing

until the solution containing no calcium and 100 per cent. citrate gave no precipitation. It will thus be seen that when approximately two equivalents of citrate were mixed with one of calcium no precipitation of the oleate solution occurred. The toxic effect of the same series of solutions when injected intravenously in mice showed a similar curve, the maximum dose of either the 100 per cent. calcium or the 100 per cent. citrate which could be administered to a mouse weighing 20 grammes being .25 c.c. As the point was approached at which no precipitation of oleate occurred, doses ranging from 2 c.c. to 4 c.c. could be injected intravenously, and at a critical point representing approximately 35 per cent. calcium it was not found possible to exert any appreciable effect upon the animals even by doubling the concentrations of the solutions employed. The effect exerted by the same mixtures of calcium and citrate upon the process of hemolysis of blood corpuscles by means of complement and amboceptor exhibited a similar curve. The solutions containing 100 per cent. calcium with no citrate and 100 per cent. citrate with no calcium entirely prevented hemolysis in the presence of an amount of amboceptor and complement four times that required to produce hemolysis in a normal system. With the decreasing proportion of calcium in the first case and citrate in the second, hemolysis took place more rapidly and at a critical point lying between 35 per cent. and 38 per cent. of calcium the interference with hemolysis was practically negligible. It will be seen, therefore, that the point at which oleate is not precipitated from its suspension in water corresponds almost exactly with that at which no disturbing effect is exerted on mice, or the process of hemolysis. At this critical point calcium and citrate are present in a ratio of approximately one chemical equivalent of calcium to two of citrate or three molecules of calcium chloride to four of trisodium citrate. This close coincidence lends support to the view that positive ions exert their protective effect in biological systems by counteracting the destructive effect of negative ions on a continuous or external lipoid phase of protoplasm in a manner similar to that outlined in a subsequent paper dealing with anesthetics.

3 (820)

On the rôle played by antagonistic ions in the process of blood coagulation.

By G. H. A. CLOWES and F. WEST.

[*From the Biological Chemical Department of the State Institute for the Study of Malignant Diseases, Buffalo, N. Y.*]

Citrated plasma prepared by admixing three parts of blood with one of 2.38 per cent. sodium citrate and centrifuging, coagulates almost immediately when admixed with an amount of $CaCl_2$ chemically equivalent to 4/10 to ½ of the citrate present. If additional citrate is added to the plasma, it is necessary to proportionately increase the amount of calcium added to induce coagulation. With thrombin, however, coagulation of plasma takes an entirely different course, the velocity of coagulation is considerably slower, but is apparently entirely uninfluenced by an excess of citrate. It may be concluded, therefore, that calcium induces coagulation by liberating thrombin from the blood platelets or other cells present in suspension in the plasma. To throw further light on this point, equal volumes of the same citrated plasma were precipitated by means of a considerable excess of a mixture of acetone and ether (an agent which has proved of considerable value in the preparation of thrombin) an excess of calcium chloride being added in one case immediately before and in the second case immediately after precipitation. The mixtures containing acetone ether were evaporated in vacuo at room temperature, the residues taken up with water and added to further amounts of citrated plasma to which an excess of citrate had previously been added. In the first case in which the calcium chloride was added before precipitation, coagulation took place rapidly, indicating the presence of free thrombin. In the second case in which the calcium was added after precipitation, no coagulation took place on addition of plasma, from which it must be concluded that by the process of precipitation the cells containing the thrombin have been rendered resistant to the action of calcium. Since the acetone-ether mixture removes not only water but fats, and since the precipitated material re-suspended in

water with the addition of $CaCl_2$ no longer produces thrombin, it must be concluded that the removal of the fatty portion of the cell membranes has rendered them non-sensitive to calcium, a point of view which harmonizes with the experiments on aqueous oil systems reported in the previous papers. As a further proof that thrombin is derived from cells suspended in the plasma which remain intact so long as the proportion of citrate to calcium exceeds 2.5 : 1, we followed the procedure recently adopted by Cramer[1] in studying oxalate plasmas, and filtered the citrated plasma through a bougie before the addition of calcium. It was found that the filtered citrated plasma was easily coagulated by thrombin but could not be precipitated by an excess of calcium even after a period of 24 hours. The residue on the filter was washed with salt solution containing a small amount of citrate, was then washed back by means of water pressure, and the aqueous suspension thus obtained divided into two parts. One part was treated with a slight excess of calcium, and when added to filtered plasma containing a large excess of citrate immediately caused coagulation from which it must be concluded that thrombin had been liberated by the calcium from cells incapable of passing through the bougie. The second portion was divided into two parts both of which were precipitated by means of acetone-ether following the procedure outlined above, the calcium chlorid being added in one case before and in the other case immediately after the addition of the acetone ether. The same results were obtained as in the previous case. The portion treated with calcium before precipitation rapidly coagulated filtered citrate plasma containing an excess of citrate. The second portion precipitated after the addition of calcium, exerted absolutely no effect upon the citrated plasma. From these experiments we are justified in concluding that thrombin is contained in blood platelets and possibly leukocytes suspended in the plasma, that the addition of a sufficient proportion of calcium to disturb the colloidal equilibrium of the lipoids in the cell membranes brings about the liberation of the thrombin, which in its turn causes precipitation of the fibrinogen, this later process being presumably entirely independent of calcium since it takes place rapidly in the presence of a large excess of

[1] *Quarterly of Exper. Physiology*, Vol. VI, p. 1.

citrate. It should be particularly noted that the ratio between calcium and citrate is approximately the same as that observed in the previous experiments on coagulation of oleate, toxicity to mice and interference with complement hemolysis, a fact which lends further support to the theory that the liberation of thrombin is associated with a disturbance in the colloidal equilibrium of fatty substances present in the cell membrane.

These experiments prove, furthermore, that the membrane contains substances other than fats and lipoids and, in the absence of the latter, is apparently uninfluenced by the addition of an excess of calcium to the system. In the light of these experiments, it should be possible to greatly simplify the existing theories regarding blood coagulation and to reduce the whole question to one of the liberation of thrombin as a result of a disturbance in the colloidal equilibrium of the platelet membrane under the influence of electrolytes and the subsequent precipitation of fibrinogen by adsorption of thrombin.

4 (821)

On analogous effects exerted by anesthetics in physical and biological systems.

By G. H. A. CLOWES.

[*From the Biological Chemical Department of the State Institute for the Study of Malignant Diseases, Buffalo, N. Y.*]

Lillie has recently demonstrated that anesthetics used in certain concentrations are capable of functioning in a manner similar to calcium salts, protecting *Arenicola* larvæ from the destructive effect of pure salt solutions. Since calcium, on the one hand, and anesthetics, on the other, are capable of rendering a concentration film of fatty acid salts or lipoids relatively more soluble in oil and less soluble in water, it appeared possible that the agents in question protect the cell protoplasm by counteracting the destructive effect of negative ions on similar surface films formed between an external lipoid phase of protoplasm, and adjacent aqueous phases. If this theory were correct it should be possible to counteract the effect of negative ions in purely physical

systems by means of anesthetics and to obtain curves corresponding with those observed by Lillie on *Arenicola* larvæ.

To test this question an aqueous solution of NaOH of suitable concentration was delivered from a Traube stalagmometer into olive oil with and without the addition of various anesthetics at varying concentrations. The number of drops formed from a given volume of solution served as an index of the extent to which the anesthetic inhibited or promoted the destructive effect of the OH ions on the film of fatty acid salts formed at points of contact between water and oil. In all cases the curve obtained showed with increasing proportions of anesthetics, first a fall in the number of drops, and subsequently a rise, the number of drops finally exceeding that of the system employed. The fall in the number of drops indicating protection of the membrane reached a maximum with each anesthetic at approximately that concentration at which Lillie observed a maximum protective effect for *Arenicola* larvæ. Furthermore on increasing the proportion of anesthetic above this optimum, the number of drops increased and finally passed the normal at approximately that point at which Lillie found no further protective effect for larvæ. The curve indicating the effect of $MgCl_2$ corresponded almost exactly with that of an organic anesthetic, but $CaCl_2$, instead of exhibiting an optimum, was found to counteract the destructive effect of OH ions not only at low but also at high concentrations, and also to exert a protective effect against Mg and organic anesthetics used at high concentrations. The accompanying diagram illustrates the effect of propyl alcohol on this purely physical system, and on *Arenicola* larvæ.[1]

C_3H_7OH.	Drops.	Larvæ.
nil.....	52	Killed.
2½ per cent.....	18	Uninjured.
10 per cent.....	76	Killed.

As a further confirmation of this point, it was found that propyl alcohol added in suitable proportions to a solution of $n/10$ NaOH would protect mice from a lethal dose of this substance injected intravenously, and furthermore that it was possible to protect to a certain extent corpuscles suspended in salt solution from the destructive effect of negative ions by the addition of optimum

[1] See Lillie, *Am. J. of Physiology*, 1913, 31, p. 255.

proportions of certain anesthetics. The close relation between curves obtained in purely physical systems and those observed by Lillie on *Arenicola* larvæ suggests the probability that anesthetics exert their effect on the cells by increasing the solubility in a fatty or lipoid phase and decreasing the solubility in the water phase of the film formed by contact between the fatty or lipoid phase and adjacent internal and external aqueous phases. If protoplasm be considered for the moment as an aqueous lipoid system in which the lipoid phase is continuous or external (a by no means impossible contingency in view of the preparation of emulsions containing 99 per cent. or more of the dispersed phase), and the water phase internal, it will easily be seen that anesthetics functioning in the manner indicated above would tend to promote the continuity of the external lipoid phase, protecting it from the destructive effect of negative ions present in the adjacent aqueous phases. In the absence of protective kations or anesthetics, negative ions adsorbed on this film or membrane would presumably induce a series of spherical curvatures with consequent contraction in length. Ultimately the film in question would be broken up into globules which would now constitute the dispersed phase. The original interior aqueous phases by uniting with the exterior aqueous medium would afford a continuous aqueous phase which would permit of the ready diffusion of any water-soluble substances originally present in the interior aqueous phase. Lillie particularly notes that *Arenicola* larvæ show violent muscular contractions, separation of fatty globules and the diffusion of water-soluble pigments into the surrounding water when subjected to the influence of pure salt solution. These effects are entirely inhibited when suitable proportions of calcium salts or anesthetics are employed.

From the above considerations it appears possible that protoplasm is an aqueous fatty system, and that anesthetics function by promoting the continuity of an external fatty or lipoid phase. The solubility of this lipoid film in adjacent aqueous phases being lowered, its permeability to water-soluble substances would be diminished. Since certain vital processes presumably depend upon intermittent intercommunication between adjacent aqueous phases, it may well be imagined that a temporary interruption in this communication would result in anesthesia.

5 (822)

The pressure changes in the right ventricle studied by optically recording manometers.

By CARL J. WIGGERS.

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

Before it is possible to interpret the inspiratory and expiratory changes in the shape and amplitude of right ventricular pressures in the unopened chest, it is necessary to know the correct contour of the pressure curve during normal cardiac cycles uninfluenced by respiration. Inasmuch, however, as the recent studies of Straub,[1] Piper[2] and C. Tigerstedt (left ventricle only[3]) by optically recording manometers reopened the old question as to the existence of a plateau or rounded top in the ventricular pressure curve, an attempt was made not only to reinvestigate the contour of the curve but to explain the causes of the different records obtained.

The changes of right ventricular pressure were, therefore, studied in open chest experiments (in which a right auricular pressure equal to intrathoracic was maintained) by inserting a sharp cannula of an optical manometer with high vibration rate through the musculature near the base of the right ventricle. The instrument and method were demonstrated.

The results show that the curve obtained depends, in a large measure, on the sensitiveness and periodicity of the manometer used. (1) With a manometer, periodic for the pressure change involved, all the details described by C. Tigerstedt for the left ventricular curve appear, viz.: an auricular wave, vibrations due to closure of tricuspids, superimposed waves on ascending level, broad, declining top, semilunar vibrations, rapid fall. (2) When the manometer becomes damped so as to become approximately aperiodic, the smaller vibrations on the ascending and descending limb are entirely lost or only faintly indicated. (3) Although

[1] Straub, *Arch. f. d. ges. Physiol.*, 1911, CXLIII, p. 69.

[2] Piper, *Zentralbl. f. Physiol.*, 1912, XXVI, No. 10, p. 429; *Arch. f. Physiol.*, 1912, p. 343.

[3] C. Tigerstedt, *Skan. Arch. f. Physiol.*, 1913, XXVIII, p. 37.

many variations of detail occur every record gives evidence of more than an evenly rounded top. During the period of cardiac ejection, the curve rises slowly and reaches a rounded summit and the wave slowly declines until the movement of cardiac relaxation, after which a sudden and abrupt fall occurs. When curves are written with too sensitive manometers the flattened top is less apparent to the eye, while, if also written on slowly moving paper as was done by Straub, this top fades to a mere suggestion requiring careful scrutiny to detect.

The results, therefore, corroborate the work of Piper and C. Tigerstedt that, during the systole of normal beats, a more or less flattened top (plateau ?) occurs while the records of Straub are not clearly typical because they were too large for the pressure change and written on too slowly moving paper. With periodic manometers vibrations are superimposed on the ascending and descending limbs but these are either lost when the manometer is aperiodic or so sensitive that a very large record is written.

6 (823)

A comparative study of the Ehrlich and Salkowski tests for indol production by bacteria.

By I. J. KLIGLER.

[*From the Department of Public Health, American Museum of Natural History.*]

One of the bio-chemical reactions extensively used for the identification of bacterial types is the production of indol from peptone in a peptone-water solution. Of the various tests that might be used for the detection of that substance the one most widely adopted in this country is the so-called Salkowski test ($H_2SO_4 + KNO_2$). In Germany Bohme (1905) and in England Marshall (1907) have found, after a comparative study, that the Ehrlich reaction (Paradimethylamidobenzaldehyde + HCl) gives more uniform and constant results.

This study was undertaken in order to obtain further light on the relative reliability of the two tests. Seventy-five organisms,

representing the various members of the colon-typhoid group, were used. Duplicate peptone broth tubes of each strain were incubated at 37° C. for four days. The contents of the tubes was then divided in half and the half portions subjected respectively to the two tests.

Twenty-eight of the seventy-five strains were positive in both duplicate tubes with both tests. Thirty-seven were uniformly negative with both tests. Five were positive in both controls with the Ehrlich test and positive in one tube and negative in the other with the Salkowski. Finally five tubes were uniformly positive with the Salkowski and negative with the Ehrlich test. The five cases in which the Salkowski test was positive and the Ehrlich test negative illustrate the fact brought out by Bohme and Marshall that the Salkowski test may give erroneously positive results when indol is not present. This error seems to be due to some red substance other than nitroso-indol but which may easily be mistaken for it. If the test is carefully performed this reddening can, however, easily be distinguished from the nitroso-indol red by the fact that it diffuses rapidly throughout the entire tube instead of remaining as a ring between the two liquids. This characteristic coloration was obtained in all those cases which gave repeated negative results with the Ehrlich test, and unlike the nitroso-indol red it was found to be insoluble in chloroform. The five other aberrant results in which the Ehrlich test was positive and the Salkowski test positive in one tube and negative in the other illustrate the possibility of an error in the other direction. I am inclined to think that the negative Salkowski tests in this case were perhaps due to a rapid oxidation of the red coloring matter. On the whole it is quite evident that the Ehrlich test is the more reliable of the two and should displace the other.

An interesting phenomenon in connection with the Ehrlich test, first called attention to by Seidelin, was also observed by me. This consists in the appearance of a purple to blue color, on the addition of the reagents, which unlike the indol red is insoluble in chloroform. Lewis, working with Seidelin, attributes a special significance to this reaction and reports three distinct colorations:

1. Soluble and insoluble red.
2. Soluble red and insoluble purple or blue.
3. No soluble red but insoluble blue or purple.

Lewis's soluble red color is due to the indol substance. I have not met with any insoluble red pigment in my tests with the Ehrlich method. The purple or blue coloration appears to be independent of indol production, as indicated by the following observations:

1. If the tubes are shaken up with chloroform without the addition of persulphate the supernatant liquid is colorless, but on standing gradually assumes a purplish and eventually either a purple-blue or blue color. Often of two duplicate tubes one was purple-blue, the other blue.

2. The addition of a few drops of fuming nitric acid or hydrogen peroxide (oxidizing agents) to the decanted supernatant liquid produces instantly the same changes in color observed gradually on long exposure.

3. The blue color reaction was obtained in uninoculated controls and in solutions of peptone in distilled water which have been treated with the aldehyde and concentrated hydrochloric acid. This shows that this coloration is independent of the indol reaction.

Since the aldehyde and acid alone do not give this reaction it is apparent that the coloration must be due either to the peptone or to some of the substances present in the peptone mixtures.

7 (824)

The influence of butter-fat on growth.

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

[*From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Connecticut.*]

When young rats are fed on mixtures of isolated food substances and inorganic salts such as the "protein-free milk" foods earlier described by the authors,[1] they cease sooner or later to grow and

[1] Osborne and Mendel, "Feeding Experiments with Isolated Food-Substances," Carnegie Institution of Washington, Publication 156, Parts I and II, 1911; "The Role of Different Proteins in Nutrition and Growth," *Science*, XXXIV, pp. 722–732, 1911; "Feeding Experiments with Fat-Free Food Mixtures," *Journ. of Biol. Chem.*, XII, pp. 81–89, 1912; "Beobachtungen über Wachstum bei Fütterungsversuchen mit isolierten Nahrungssubstanzen," *Zeitschr. f. physiol. Chem.*, LXXX, pp. 307–370, 1912.

they then decline upon these diets. Milk food speedily brings restoration of growth; and it has been shown that the "essential" accessory factor responsible for this effect is a component of the cream which is present in butter.[1] Further experiments now indicate that the butter-fat separated by centrifugal methods from unsalted butter contains the substance which averts the cessation of growth and possible nutritive decline noted when lard is used instead of milk-fat.

Butter-fat thus prepared is free from nitrogen, phosphorus and ash-yielding constituents. The growth-promoting substance therefore is not a phosphatide (lecithin) or an inorganic compound.

8 (825)

The presence of creatinine in muscle.

(PRELIMINARY PAPER.)

By MORRIS S. FINE and VICTOR C. MYERS.

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The presence of creatinine in muscle has, in general, been denied by those who have undertaken a study of this question. In earlier communications[2] upon various phases of the creatine-creatinine problem, evidence has been presented which is strongly in harmony with the metabolic relationship of these two substances. Since creatinine is so rapidly eliminated from the body, its presence in the muscle tissue would not be expected in large quantity. On the other hand, if creatinine originates from creatine, this transformation might be expected to take place in the muscle tissue, and on this account it would seem that, with sufficiently delicate and reliable methods, it ought to be possible

[1] Osborne and Mendel, "The Relation of Growth to the Chemical Constituents of the Diet," *Journ. of Biol. Chem.*, XV, pp. 311–326, 1913; also McCollum and Davis, "The Necessity of Certain Lipins in the Diet During Growth," *Journ. of Biol. Chem.*, XV, pp. 167–175, 1913.

[2] See Myers and Fine, PROC. SOC. EXP. BIOL. AND MED., 1912–13, X, pp. 10, 12 and 168; also *Jour. Biol. Chem.*, 1913, XIV, p. 9; XV, pp. 283 and 305; XVI, p. 169.

to detect the presence of creatinine. We believe we have demonstrated that creatinine does exist in very small quantity in muscle (rabbit), equivalent to about one per cent. of the creatine. The quantity appears to average 6 or 7 mgm. per 100 grams of moist muscle, although variations of 3 to 10 mgm. have been encountered. An observation which is much more significant, however, is that, when the muscle is allowed to autolyze at body temperature under antiseptic conditions, the creatinine content increases at a very uniform rate at the expense of the muscle creatine. One experiment may be cited. The muscle of Rabbit No. 77 had an initial creatinine content of 6 mgm. per 100 grams; at the end of 2 days this had increased to 20.2 mgm., in 4 days to 36.6 mgm., in 6 days to 50.6 mgm., in 8 days to 62.5 mgm., and in 10 days to 74.5 mgm., an average *uniform daily increment* of 7 mgm. per day. This fact, when considered in connection with the uniform content of muscle creatine previously observed by us,[1] is, we believe, the fundamental factor in bringing about the constant daily excretion of creatinine originally observed by Folin. When pure creatine is added to autolyzing muscle it experiences the same fate as the creatine originally present, while in one experiment where creatinine was added in an amount equivalent to the creatine present, it was found to inhibit the usual transformation. This would seem to indicate that the reaction between these two substances is reversible.

The method employed for the determination of creatinine in muscle is briefly as follows: The finely ground muscle tissue is thoroughly extracted with cold water, and to the extract sufficient alumina cream is added to precipitate the proteins (and enzymes). The mixture is then made up to volume, filtered, and a portion of the perfectly clear filtrate is evaporated with the aid of an electric fan to a sufficiently small volume to make the usual colorimetric estimation.

The present preliminary experiments are the beginning of a series which are planned upon this problem.

It is suggested that the above method for creatinine may be of service commercially in ascertaining the age of meat.

In connection with the present work we have observed that

[1] *Loc. cit.*

certain dietary factors (referable to the nature of protein) exert an influence upon the content of muscle creatine. This observation is being further investigated.

9 (826)

On re-injection with B. tuberculosis or its products and with sera.

By J. P. ATKINSON and C. B. FITZPATRICK, M.D.

[*From the Chemical and Research Laboratories, Department of Health, City of New York.*]

We have stated in several papers read before this Society that extreme vaso-depression was caused by the intravenous injection of split products of bacterial and other origin. These statements were part of an endeavor to elucidate experimentally the mechanism by which foreign organisms and substances may possibly cause intoxication, infection and anaphylaxis by neutralizing pressor secretions, by removing or using up the nourishment of the host, or by destroying the processes upon which depend the host's specificity, vaso-energy and power of reforming foreign bodies into substances like its own constituents, which may be essential in these conditions to its existence.

This view includes the action of those foreign substances and ferments upon the host, by which the host organism or substrata gives off split products which thus produce an auto-intoxication.

These notes carry this view still further by means of a few observations on death and symptoms following re-injection in animals which have already been injected with tubercle bacilli or products of the tubercle bacilli and with sera. An explanation is advanced that the death which followed the re-injection of the minute dose of *B. tuberculosis*, with the long interval between the first and second injection, namely twelve months, is probably due to a deferred anaphylaxis or persistent increased susceptibility or sensitiveness which may be present for an unusually long time in some of the cases which have apparently recovered from the first injection. There were not enough tuberculous lesions found in this case to satisfactorily account for death.

The initial dose according to this interpretation produces a state of responsiveness or sensitiveness which reacts to the attack or action of the re-injection, by the production of free-bonded substances and split products, poisonous secretions or eliminations which result in the intoxication or functional disturbance which may end in death.

A practical application of our observations is made to the administration of therapeutic sera. The re-injection of split products of the *B. tuberculosis* obtained by chemical agents and electrolysis have given interesting results.

PROTOCOL 1: A POSSIBLE DELAYED ANAPHYLAXIS FOLLOWING RE-INJECTION WITH *B. tuberculosis*.

Large black mongrel dog, weight 25 lbs., was injected April 25, 1912, intravenously with 4 mg. No. 634 bovine culture of the *B. tuberculosis* contained in 4 c.c. of physiological saline solution. The dog was sick for about two months, with loss of weight. It then recovered and gained weight and strength and appeared to be in good health. Nine months after this recovery and 12 after the first injection, the dog was apparently normal and had increased in strength and weighed 33¼ lbs. This dog was re-injected on April 16, 1913, with 4 mg. intravenously of a virulent bovine culture (Ravenel) of the *B. tuberculosis*, contained in 4 c.c. of physiological saline solution. This dog appeared well immediately after the re-injection and remained so for ten days. Twelve days after the re-injection bloody mucus stools were observed. It then became quiet and three days later, 17 days after the re-injection, it was found dead with some signs of hemorrhage from the nostrils. The autopsy showed a very few tubercles in the pleura covering the upper lobes of the lungs. The liver was fatty and the lungs extremely congested. The spleen and other organs were negative. The cause of death in this case would possibly appear to be a persistent or delayed constitutional reaction, rather than due to the 4 mg. of the tubercle bacilli per se. The initial dose set up a condition in the host which responded to the re-injection by setting free ferments and split products or secretions, which thus produced an auto-intoxication as outlined in the beginning of this paper.

Control: Small white dog, 12 lbs., received April 25, 1912, an initial injection of 4 mg. of bovine culture No. 634, intravenously in 4 c.c. of physiological saline solution. The dog was sick and lost weight for several months, and then regained its weight. Two superficial abscesses with necrosis of the skin and subcutaneous tissue developed at the point of inoculation, which healed after a few months. This dog was re-injected intravenously April 16, 1913, with 4 mg. of a virulent bovine culture (Ravenel) of the *B. tuberculosis* contained in 4 c.c. of physiological saline solution. The healed ulcers of the initial injection re-opened 12 days after the re-injection as two clean, punched out ulcers and an area of infiltration arose at the site of the re-inoculation. This dog became much emaciated and had a purulent conjunctivitis. No hemorrhage was observed from nostrils or in stools. Respiration 48 per minute, 19 days after the re-injection. Died May 12, 1913. Autopsy showed a typical general miliary tuberculosis, with mediastinal glands enlarged and caseous. The cause of death in this dog was undoubtedly general tuberculosis.

PROTOCOL 2: CONTROLS ON CULTURE NO. 634 (BOVINE).

Mongrel dog, 25 lbs., injected intravenously April 25, 1912, with 4 mg. No. 634 bovine culture of *B. tuberculosis*, was killed June 26, 1912. The autopsy showed a case of typical infection. The lungs contained numerous small tubercles, with no caseation or consolidation. Kidneys, about 300 large tubercles. Liver, a few tubercles. Several other dogs were used to control the bovine cultures, Ravenel and No. 634.

PROTOCOL 3: DELAYED ANAPHYLAXIS.

A small quantity of the insoluble non-toxic portion of the tubercle bacilli, prepared according to Vaughan's method, was injected into each of 2 guinea pigs. After 3 weeks, these two pigs were re-injected, intraperitoneally, with an emulsion of tubercle bacilli, from which the split products had been prepared.

Pig No. 1 showed some restlessness, at the time of re-injection, but gave no other sign that it had been affected by the re-injection. Two days later this pig was seized with a convulsion and died.

Control Pig No. 2: Re-injected as above. 30 minutes after

the re-injection, restlessness followed by weakness in the hind legs; violent convulsions followed by extreme fatigue. The convulsions continued and death followed in a convulsion six hours after the injection.[1]

PROTOCOL 4: RE-INJECTION WITH TUBERCULOUS SERUM FOLLOWING A TUBERCULIN INJECTION.

The subcutaneous injection of 10 c.c. of the serum of a tuberculous rabbit into each of three rabbits, each of which had been previously sensitized by an injection of 1 c.c. of crude tuberculin, caused death in 24 to 50 hrs.[2]

The autopsy showed, as the principal lesion, large, marked areas of intestinal inflammation and necrosis.

If this state is dependent on substances with free bonds or not homogenized, or on split products, as we have outlined, one should be able to detect it by the presence of reactions, changes of blood pressure, precipitins, ferments or similar agents, loose in the fluids or stored in the organs.

It has been shown by one of us that tubercle bacilli suspended in a saline solution are considerably changed upon being electrolyzed in their reaction to stains. If a porous cell such as a Berkfeld filter is used as a septum in a U-tube and suspensions of tubercle bacilli are put in both arms, one portion will be acted upon by chlorine and the other portion by sodium or sodium hydrate as a result of electrolysis. The organisms acted upon by chlorine lose their acid-fast quality when decolorized by nitric acid and substances that have the acid-fast principle may be shaken out of solution with ether. The portion acted upon by sodium or sodium hydrate does not noticeably lose its acid-fast principle. There are other changes which can be demonstrated and which are now being investigated.

A guinea pig was re-injected twice with tubercle bacilli which had been submitted to the above chlorine treatment. The injections were made during a period of two weeks. Within an hour after the third injection the guinea pig showed signs of illness and became steadily worse during the day. It died during the night

[1] A. and F., PROC. OF SOC. FOR EXP. BIOL. AND MED., 1910, VIII, pp. 24–28.
[2] A. and F., PROC. OF SOC. FOR EXP. BIOL. AND MED., 1910, VII, pp. 77–79.

following the third injection. Autopsy showed inflammation and extensive necrosis at the point of injection.

There were no characteristic signs of anaphylaxis observed during the illness of this guinea pig, although this was probably a case of death after sensitization. The electrolytic products of the tubercle bacillus are being further tested as possible immunization agents.

The variable degree of the persistence of sensitiveness in and following infections has led us in the use of sera to inject a very minute initial dose of serum and to repeat with a slightly increased dose instead of using one large dose of the entire quantity to be administered. This method was elaborated by us as shown before this society.[1]

10 (827)

Some observations on the action of ergot.

By WILLIAM SALANT and C. T. HARRIS.

[*From the Pharmacologic Laboratory, Bureau of Chemistry, Washington, D. C.*]

The activity of ergot was tested by the cock's comb reaction on white Leghorn roosters, the fluid extract being employed in all the experiments. Alcohol was found to increase the susceptibility to the drug as shown by the decreased activity of the preparation after the alcohol was driven off. Smaller doses of ergot were also required to produce a reaction in acute alcohol intoxication. A fall of temperature was also noticed when ergot was given in this condition, thus indicating a reversible action, since in the normal subject the injection of ergot frequently caused a marked rise of temperature. The repeated injection of ergot at intervals of 24 to 48 hours failed to produce any cumulative effect in the normal subject. But in starvation there was a decided bluing of the comb after the third injection of a sub-minimum dose, the effect becoming more marked with successive injections. In a large number of experiments carried out on normal subjects it was found that when the injections are made at proper intervals the cock's comb reaction

[1] PROC. OF SOC. FOR EXP. BIOL. AND MED., Feb. 21, 1912, IX, pp. 49–51.

became less marked after repeated administration of the drug. Such a tolerance was observed after large doses of very active preparations of the fluid extract and also when the alcohol was driven off. Although the final amounts administered were gradually increased, six to ten times, the bluing of the comb observed was in many cases less marked than after the initial dose.

11 (828)

Creatine in the muscle tissue of the lamprey.

By D. WRIGHT WILSON and JOHN F. LYMAN.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

Creatine was isolated from the muscle tissue of the lamprey, *Petromyzon marinus*.

The lampreys, commonly known as "lamprey eels," are sea animals found on our shores, and belong to the lowest class of vertebrates, the Cyclostomata. For this reason, the presence of creatine in the muscle extracts is of especial interest. It has never been isolated from the muscles of invertebrates and its occurrence in this, the lowest form of vertebrate, seems indicative of some radical and sudden difference in the composition of the muscle tissue between these two great animal divisions.

Mellanby[1] estimated the creatine content of lamprey muscle by Folin's colorimetric method but failed to isolate the compound. Isolation is necessary for proof in muscle extracts of these low forms where anomalous color reactions often occur.

A water extract of the muscle tissue was made, freed from protein, evaporated to a small volume and allowed to stand. The creatine which separated out was purified by recrystallization and analyzed.

[1] Mellanby, *Journal of Physiology*, 1908, *36*, 472.

12 (829)

The combined effect of magnesium sulphate and sodium oxalate upon rabbits.

By F. L. GATES and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

Our communication is concerned not with the prolonged, but with the *immediate* effect of the salts under discussion. The acute death of a rabbit which may occur within one hour or two after a subcutaneous injection of twenty-five or thirty centigrams of sodium oxalate per kilogram body-weight, is often preceded by a series of convulsions which may last fifteen to twenty minutes and which evidently are not due to asphyxia. Sublethal doses of ten or fifteen centigrams, which occasionally may cause some hypersensitiveness, are not followed by any serious symptoms. On the other hand death following subcutaneous injections of magnesium sulphate is ushered in, according to Meltzer and Auer, by paralysis without convulsions. Two grams of the salt (+ crystalline water) is a surely fatal dose; one gram and a half per kilo body-weight usually causes anesthesia and paralysis followed by recovery. Doses of less than one gram per kilo body-weight may cause a more or less definite state of drowsiness and weakness which is generally of short duration only. In no case of magnesium poisoning are the narcotic and paralytic symptoms complicated by convulsions or hyperesthesia.

The results which we have obtained in experiments with the simultaneous injections of both salts can be stated briefly as follows. *Simultaneous injections of small doses of magnesium sulphate and sodium oxalate in separate parts of the animal body seem to produce an effect equal to that of a larger dose of magnesium sulphate alone.* When, for instance, twelve or fifteen centigrams (per kilo body-weight) of sodium oxalate are injected subcutaneously into one side of the rabbit and eight or nine decigrams (per kilo body-weight) of magnesium sulphate are injected into the other side, the result is the development of such a degree of

complete anesthesia and paralysis as follows a subcutaneous injection of fifteen decigrams of magnesium sulphate alone. In other words the result of the injection of subminimal doses of sodium oxalate and magnesium sulphate is a greatly intensified effect, which, however, does not seem to correspond to a combination of the divergent effects of the two salts but corresponds rather to a summation of two subminimal doses of magnesium salt alone. There was this difference, however: the state of anesthesia and paralysis produced by the injection of the two salts lasted definitely longer than is usual after an injection of an effective dose of magnesium alone.

The results were constant; except for one or two failures at the beginning, the outcome of every experiment was in the direction stated above.

The experiments were undertaken on the basis of considerations which follow from the observations of Meltzer and Auer on the antagonistic action of calcium to magnesium, namely that the anesthetic and paralytic action of magnesium is rapidly reversed by an injection of calcium. As a consequence of this fact, it seemed probable that a reduction of calcium within the body might be capable of augmenting the depressing action of magnesium. Now we know that oxalates precipitate calcium salts in crystalloid solutions and also antagonize their effects in animal fluids, as is illustrated in the process of fibrin formation. It was therefore considered possible that oxalates, by reducing in some degree the calcium action within the body, might increase the effect of subminimal doses of magnesium. The results of our experiments seem to support this hypothesis.

13 (830)

The effects of protein starvation and feeding on the amino-acid content of the tissues.

By DONALD D. VAN SLYKE and GUSTAVE M. MEYER.

[*From the Laboratories of the Rockefeller Institute for Medical Research.*]

Starvation for periods up to twelve days does not reduce the amino-acid content of the tissues of dogs, nor does high protein feeding (500 grams of beef daily added to the regular diet for 1 to 7 days) increase it. The results indicate that:

1. Nitrogen retained as the result of high protein feeding is not in the form of stored digestion products, but rather as body protein.

2. The free amino-acids of the tissues can originate not only from the food, but also from autolysing tissues, as the latter are the only apparent source from which the amino-acid supply can be maintained during starvation.

14 (831)

Hibernation and the pituitary body.

By HARVEY CUSHING and EMIL GOETSCH.

[*From the Department of Surgery, Harvard Medical School, Boston, Mass.*]

A train of symptoms, coupled with retardation of tissue metabolism and with inactivity of the reproductive glands, not only accompanies experimental states of hypophysial deficiency but is equally characteristic of clinical states of hypopituitarism. The more notable of these symptoms are a lowering of body temperature, slowing of pulse and respiration, fall in blood pressure, and somnolence, together with a tendency, in the chronic cases, toward an unusual deposition of fat.

These symptoms are comparable to those accompanying the state of hibernation.

In a series of hibernating animals (woodchucks) it has been found that during the dormant period the pituitary gland not only diminishes in size but undergoes extreme histological alterations, chiefly evident in the cells of the pars anterior, which completely lose their characteristic differential reactions to acid and basic stains. At the end of the dormant period the gland enlarges and the cells regain their characteristic staining reactions.

On the basis of this observation hibernation may be ascribed to a period of physiological inactivity, possibly of the entire ductless gland series, but certainly more especially of the pituitary body, not only for the reason that the changes in this structure are particularly apparent but because deprivation of the secretion of this gland alone of the entire series produces a train of symptoms comparable to those of hibernation.

15 (832)

The pars anterior and its relation to the reproductive glands.

By EMIL GOETSCH and HARVEY CUSHING.

[*From the Department of Surgery, Harvard Medical School, Boston, Mass.*]

We wish to report briefly some of the results obtained thus far by the feeding of dried extract of bovine hypophysis to young rats of pure breed and known pedigree. The animals were taken as soon as weaned at the age of three or four weeks. In each instance the experiments were controlled by observations on other rats of the same litter, kept under similar conditions without grandular administration. A known weight of dried extract (.1–.05 gm.) was given daily in a bread and milk pill, and over varying lengths of time, after which the animals were sacrificed and the reproductive glands examined. Both anterior and posterior lobe extracts were used and in several instances ovarian or corpus luteum extract was given in equal dosage to the control animals, to rule out the effect of administration of glandular extracts in general.

In a limited number of instances pairs of young rats of the same

litter were taken, one pair being given small quantities of extract of pars anterior, the other pair used as a control. The animals fed with the extract bred sooner and oftener than the controls. Posterior lobe extract of pituitary gland had no such effect.

Conclusions.—The following conclusions seem warranted:

I. Pituitary extract, and particularly extract of pars anterior, has a markedly stimulating effect upon the growth and development of the reproductive glands in young rats of both sexes, as evidenced by histological examination.

II. Extract of pars anterior tends to cause early and frequent breeding. Posterior lobe extract has no such effect.

III. Posterior lobe and ovarian (corpus luteum) extracts apparently do not stimulate sexual development.

16 (833)

On the correlation between the number of mammæ of the dam and size of litter in mammals. I. Interracial correlation.

By RAYMOND PEARL.

[*From the Biological Laboratory of the Maine Agricultural Experiment Station.*][1]

In his breeding experiments with sheep at Beinn Breagh, Alexander Graham Bell[2] found that as the number of nipples of the ewes increased there was a tendency towards a more frequent production of twins and triplets. Regarding this point he says (*loc. cit.*, p. 383): "The indications are that our six-nippled stock will ultimately turn out to be twin bearers, as a rule, when they become fully mature."

In reporting a case of unusually high, and probably inherited, fertility in the cow I have[3] noted that the individual exhibiting this high fertility bore two supernumerary mammæ.

There is an obvious teleological aspect to this matter. In a general way it is clear that as the number of young born in a litter increases there must be a compensating increase in the number of

[1] Paper No. 52.

[2] *Science*, N. S., Vol. 36, pp. 378–384, 1912.

[3] Me. Agr. Exp. Sta. Ann. Rept. for 1912, pp. 259–282.

mammæ, unless there is some peculiarity of feeding habits in the young which would nullify the advantage, not to say necessity, of having enough nipples to "go around." It would seem, *a priori*, that natural selection should have operated to bring about a high correlation, both intra- and interracial between these two variables, size of litter and number of mammæ of the dam. The purpose of this paper is to present the results of a study of interracial correlation between number of mammæ and size of litter.

DATA.

The most extensive series of statistics regarding size of litter and number of mammæ which I have been able to find is that given by Bellingeri[1] in a treatise on "Mastologia."

Bellingeri gives (*loc. cit.*, p. 84) a table including something over a hundred different species of wild and domestic mammals, representing all the orders of Mammalia, in which so far as possible the following information is given for each species: number of mammæ in the female, number of young born at a birth, breeding season, and certain data respecting breeding and feeding habits. So far as it has been possible to check the records against more modern work they have been found to be in general correct. In using the material all doubtful and incorrect cases which could not be corrected from available literature have been eliminated. Further there are a certain number of species for which information on one or more of the points tabled is lacking. After all these eliminations there are left 90 species for which there are complete (so far as concerns the present problem) and presumably accurate records.

In dealing with the material biometrically it has been necessary to make certain assumptions. In a number of instances the figures given for both number of young and number of mammæ are the limits of the range of intraracial variation rather than the intraracial mode, mean or median. In such cases it is necessary to make an approximation to the intraracial mean before the figures can be entered in a correlation table. In reaching these approxima-

[1] Bellingeri, C. F., "Della fecondita e della proporzione dei sessi nelle nascite degli animali vertebrati, e mastologia, con considerazione anatomico-fisiologichi sul numero e posizione delle mamelle," Tome III, Torino, 1849.

tions every endeavor has been made to get as near the actual fact as possible. When no information could be found regarding the point at issue in a particular species it was assumed that the mid-point of the range, as given by Bellingeri, could be taken as the centering point, with sufficient accuracy. It is reasonable to suppose that any errors introduced by making this assumption will not be biased, but will as often be in one direction from the truth as in the other.

The interracial correlation table derived from Bellingeri's data is shown in Table I.

TABLE I.

SHOWING THE INTERRACIAL CORRELATION SURFACE FOR THE VARIABLES (*a*) NUMBER OF MAMMÆ, (*b*) NUMBER OF YOUNG AT A BIRTH.

Mammæ of Dam.	Size of Litter.															Totals.
	1–1.9	2–2.9	3–3.9	4–4.9	5–5.9	6–6.9	7–7.9	8–8.9	9–9.9	10–10.9	11–11.9	12–12.9	13–13.9	14–14.9	15–15.9	
2– 3.9	26	4	2	1	1	—	—	1	—	—	—	—	—	—	—	35
4– 5.9	11	—	3	—	2	—	—	—	1		—	1	—	—	—	18
6– 7.9	—	2	3	2	2	—	1	—	1	—	—	—	—	—	—	11
8– 9.9	—	1	1	2	3	—	2	1	—	—	—	—	—	—	—	10
10–11.9	—	—	2	2	2	2	—	—	2	—	—	—	—	—	1	11
12–13.9	—	1	—	—	1	2	—	—	—	—	—	—	—	—	1	5
Totals.....	37	8	11	7	11	4	3	2	4	0	0	1	0	0	2	90

TABLE II.

CONSTANTS OF VARIATION DEDUCED FROM TABLE I.

Character.	Mean.	Standard Deviation.	Coefficient of Variation.
Number of mammæ.....	6.09 ± .23	3.25 ± .16	53.45 ± 3.36
Number of young at birth....	3.92 ± .22	3.05 ± .15	77.80 ± 5.81

Coefficient of correlation $r = .594 \pm .046$.

From this table the following points are to be noted:

1. Interracially the mean size of litter is approximately two individuals below the mean number of mammæ possessed by the mother. This may be taken as a rough measure of the evolutionary "factor of safety" in regard to these characters.

2. There is relatively (cf. coefficients of variation) somewhat more variation exhibited in size of litter than in number of mammæ.

3. The correlation between these two characters, as measured by the coefficient r, is surprisingly *low*. This result certainly cannot be said to furnish particularly strong evidence that natural selection has had anything to do with fixing the relationship between number of mammæ and size of litter.

Turning next to the regressions it is found that the regression of size of litter on mamma number is sensibly linear. The more interesting and significant regression of number of mammæ of dam on size of litter is non-linear, as is shown by the following constants[1]

$$\eta = .7714,$$

$$\zeta = .2426,$$

$$\frac{\sqrt{N}}{.67449}\tfrac{1}{2}\sqrt{\zeta} = 3.412.$$

The determination of the precise form of the regression equation here cost a good deal of labor. It was finally found to be logarithmic. The following equation expresses the relation between the number of young at birth and the mean number of mammæ of the dam, the constants having been determined by graduation of the first five means of arrays. Beyond that point resort is had to extrapolation, since the number of observations is too small to give reliable results.

y (probable mean number of mammæ of dam) $= 3.9616 - .3512x$ (number of young in litter) $+ 8.6208 \log x$.

For the linear regression of size of litter on mamma number we have

x (probable number of young per birth) $= .5566y$ (number of mammæ of dam) $+ .5331$.

[1] Cf. Blakeman, *Biometrika*, Vol. IV, pp. 332–351, 1905.

17 (834)

On the correlation between number of mammæ of the dam and size of litter in mammals. II. Intraracial correlation in swine.

By RAYMOND PEARL.

[*From the Biological Laboratory of the Maine Agricultural Experiment Station.*][1]

The data here discussed regarding the intraracial correlation between mamma number and size of litter in Duroc Jersey swine were furnished me by Professor E. N. Wentworth, formerly of the Iowa State College. I take this opportunity of expressing my appreciation of this kindness. The material was collected in the course of Professor Wentworth's studies on the inheritance of mamma number.[2] In the case of 13 out of the 57 dams recorded data are available for the size of two successive litters, namely those of 1911 and 1912. In these cases, in order to avoid unequally weighting the table because of the fertility of the dams, each dam has been entered in Table I once only, the litter size entered being the mean of the two successive litters.

The ungrouped data are given in Table I.

TABLE I.

SHOWING THE INTRARACIAL CORRELATION SURFACE FOR THE VARIABLES (*a*) NUMBER OF MAMMÆ OF DAM, AND (*b*) SIZE OF LITTER, IN SWINE.

Mammæ of Dam.	Size of Litter.														Totals.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	1	—	—	1	1	2	1	3	2	4	3	—	—	—	18
11	—	—	—	—	—	3	—	1	1	—	2	—	—	1	8
12	—	—	—	—	—	—	5	4	4	3	4	1	3	—	24
13	—	—	1	—	—	—	—	—	—	1	3	—	—	—	5
14	—	—	—	—	—	1	—	—	—	—	1	—	—	—	2
Totals.....	1	0	1	1	1	6	6	8	7	8	13	1	3	1	57

In dealing with this table slightly different methods were used than in the case of the interracial correlation.[3] The classes of both variables have been assumed to center at the points given by

[1] Paper No. 53.

[2] *American Naturalist*, Vol. XLVII, pp. 257–279, 1913.

[3] Cf. these PROCEEDINGS, Vol. X, p. 31, 1913.

the actual counts. In other words it has been assumed that we are dealing here with strictly integral variates. This assumption seems justified for the present material, but not for the interracial material of the previous paper.

The constants deduced from Table I are given in Table II.

TABLE II.

CONSTANTS OF VARIATION DEDUCED FROM TABLE I.

Character.	Mean.	Standard Deviation.	Coefficient of Variation.
Number of mammæ.....	11.39 ± .10	1.12 ± .07	9.84 ± .63
Number of young at birth....	8.88 ± .23	2.54 ± .16	28.63 ± 1.95

Coefficient of correlation $r = 0.195 \pm .086$.

From this table the following points are to be noted.

1. There are approximately 2.5 more mammæ in the dam, on the average, than number of young in the litter in these swine. This is a slightly larger "factor of safety" than is found interracially.

2. The intraracial correlation between these variables in swine is not only absolutely low, but is relatively much lower than the interracial correlation. Again it is not apparent here that natural selection has operated in the expected manner.

3. Intraracially, just as interracially, size of litter is relatively a more variable character than number of mammæ in the dam.

4. There is, as would be expected, a very considerable reduction in variability, in respect of both characters, in the single species (intraracial) as compared with the composite group of 90 different species (interracial).

18 (835)

The effect of animal extracts upon the volume of the spleen.

By ISAAC OTT, M.D., and JOHN C. SCOTT, M.D.

[*Physiological Laboratory, Medico-Chirurgical College of Philadelphia.*]

We studied the volume of the spleen with an oncometer attached to a modified piston recorder. The animals employed

were etherized cats. The infusion of the extracts was injected by the jugular. The arterial tension was registered by a Hürthle manometer. The extracts increasing the size of the spleen beyond normal were adrenalin, infundibulin, corpus luteum, thymus, orchitic extract, parathyroid and iodothyrin. The extracts diminishing the splenic volume were ovary and pineal. The agent causing large rhythmical contractions of the spleen was splenic extract.

19 (836)

Metabolism studies in a case of myasthenia gravis.

By THEODORE DILLER, M.D., and JACOB ROSENBLOOM, M.D., Ph.D.

[*From the Wards of the St. Francis Hospital and the Biochemical Laboratory of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

In an eight-day metabolism study on an individual suffering from myasthenia gravis, we have studied the nitrogen metabolism and urinary nitrogen partition, the sulphur metabolism and urinary sulphur partition, and the calcium, magnesium, phosphorus and fat metabolism. The creatinin, uric acid and neutral sulphur excretions were markedly less than the normal. A considerable loss of calcium was found. The addition of 300 grammes of egg yolk to the diet caused a phosphorus retention, not accompanied by retention of either calcium or magnesium. The fat metabolism was normal.

20 (837)

A case of interpolated extrasystoles in an otherwise normal human heart, illustrated by electrocardiograms.

By M. DRESBACH and S. A. MUNFORD.

[*From the Physiological Laboratory, Cornell University, Ithaca, N. Y.*]

At the meeting of this Society on October 16, 1912, we presented a case of interpolated extrasystoles in an otherwise normal

human heart, pointing out several interesting features in the polygraphic records of the case. These records were of such a nature that interpretation of them was very difficult and led to doubtful conclusions. Since that meeting we have been fortunate in securing excellent electrocardiograms of this heart through the kindness of Dr. H. B. Williams. These records show that the interpolated beats arise constantly in the right ventricle, and probably in the right branch of the A–V bundle; the curves indicate that the abnormal impulse travels to the basal part of the left ventricle and thence to the apex, and that this path is always the same; there is no evidence of retrogression to the auricle, as was suspected from the mechanical tracings, but there is evidence of depression of the conduction system, for the P–R intervals of normal beats following the extrasystoles are often considerably lengthened (we could not be certain of lengthened As–Vs intervals in the polygrams). Phonocardiograms show divided second sounds produced by the abnormal beats and lengthened first sounds. A few compensating pauses have been seen in the electrocardiograms.

Aside from these new facts about the case, our previous report needs no alteration.

21 (838)

The incidence of cancer in various strains of mice.[1]

By A. E. C. LATHROP and LEO LOEB.

[*From the Department of Pathology, Barnard Free Skin and Cancer Hospital, St. Louis.*]

A number of investigators noticed the repeated occurrence of a particular kind of cancer in animals living either in the same cages or

[1] These investigations are the outcome of plans for research which one of us suggested about seven years ago; on that occasion we pointed out the necessity for the study of possible hereditary and infectious factors in cancer directly in breeding establishments. In accordance with these suggestions our investigations were carried out in conjunction with Miss Lathrop in Granby, Mass., one of the most extensive breeders of mice, who supplied at various times different laboratories in this country with ordinary as well as with tumor mice, and from whom we obtained about seven or eight years ago a mouse with a spontaneous tumor which we have since propagated through many generations of mice and which was used in the majority of our experiments.

on the same farm. One of us described such observations in the case of carcinoma of the inner canthus of the eye in cattle found in increased number on a farm in Wyoming and in the case of three sarcomata of the thyroid in a relatively small number of white rats kept in a few cages in the laboratory of the Chicago Polyclinic and compared this phenomenon to the so-called endemic occurrence in man, but emphasized from the beginning that we had to consider not only infectious, but also hereditary conditions as possible causative factors.

While in the case of certain cancers, for instance the squamous cell carcinoma of rats observed by Hanau, the above mentioned carcinoma in cattle and the sarcoma of thyroids of rats and some other similar cases observed by Borrel and others the unusual frequency of the reported cases in certain places could not be doubted, the significance of similar observations concerning the frequent occurrence of mammary cancer in mice were less clear, inasmuch as mammary cancer of mice is found everywhere and sufficient comparative statistics do not exist as yet as to the normal incidence of cancer in various strains of mice.[1] Our investigations were therefore concerned with the incidence of cancer among various strains of mice kept in the same breeding establishment, especially with the view to decide definitely whether or not a hereditary factor was detectible in the occurrence of spontaneous cancers in mice.

The principal results of our investigations which, after a few preliminary observations were begun more than three years ago, and which we hope to be able to continue, may be summarized as follows:

1. Mice raised in the same breeding establishment, under the same conditions of climate and feeding show a very different incidence of cancer of the mammary gland among female mice depending on the strain or family to which the mice belong. To give a few examples: The strain designated "English," 66.9 per cent.; "Carter," 35 per cent.; "No. 8," 30 per cent.; cream, 3⅓

[1] The more or less frequent occurrence of mammary cancer in mice in certain cages or breeding establishments had previously been reported among others by Eberth and Spude, Borrel, Michaelis, Gaylord and Clowes (who also observed the occurrence of sarcoma of rats in cages in which one of us had previously kept rats inoculated with sarcoma), Apolant, Ascher, Henke.

per cent.; hybrids between European and No. 10, 82 per cent.; cancer incidence.

2. The tumor rate in the different strains remains approximately constant in the succeeding generations; a few times we noticed however an apparent increase in the tumor rate after the first two generations. Also different substrains obtained through selection of certain individuals from a large strain which may or may not differ in color or in other peculiarities from other substrains, show usually a similar tumor rate as the main strain.

Thus the substrain "English tan" had a tumor incidence of 73 per cent., "English sable" 76 per cent., "English 101" 65 per cent. It is however, in some cases, possible to detach from the main strain a substrain, that differs markedly from the main strain in the tumor rate. Thus the "English silver," a substrain of the "English," has only a tumor incidence of 8⅓ per cent. In this case the factor or factors determining the origin of tumors seem to be linked with the factor or factors of the silver color.

3. Various strains of mice do not only differ in regard to the incidence of cancer, but also in regard to the age at which the cancers appear. Thus in the English strain as a whole (excluding the silver substrain) tumors appeared in 68 per cent. of the cases in mice at the age of 12 months or below, and in only 4.6 per cent. of the mice the tumors appeared above the age of 17 months, while in the strain No. 8 approximately 72 per cent. of the animals have tumors above the age of 12 months and 36 per cent. above 17 months. In the "Carter" strain the tumors appear in 63 per cent. at or below the age of 12 months and in the hybrids between European and No. 10, which have the high tumor rate of 82 per cent. the tumors appear in 69 per cent. above 12 months and in 22 per cent. above 17 months of age, therefore considerably later than in the English, who also have a very high tumor rate. In various English substrains the tumors appear therefore much earlier than in all the other strains, although the tumor incidence as a whole is not as high in the English strain as in the hybrids between European and No. 10. In valuating this fact we have however to take into consideration the usually shorter duration of life of the English in contradistinction to some other strains.

The youngest mice in which we observed tumors were 5½ months old at the time their tumors were first noticed.

4. Although in several strains the incidence of cancer increased with advancing age, in another strain (No. 8) a maximum was reached between the age of 14 and 20 months, after which again a decrease took place in the percentage of mice affected by cancer.

5. In mating a strain rich in tumors (English) with a strain very poor in tumors (cream) we received hybrids which have so far been rich in tumors. In this case the male which is itself not liable to have tumors transmits the liability to have tumors to the daughters; the latter have tumors, although they are nursed by mothers which are not liable to have tumors. We wish however to collect still further data concerning this point before we regard this conclusion as definite.

6. It is not always probable to predict from the known cancer rate of the parents the cancer rate of the hybrids. Thus No. 10 mated to a strain called "European" gave hybrids with a tumor rate of 82 per cent., while No. 10 mated to No. 8 seems to give a very low tumor rate, although pure Europeans had a lower rate (13 per cent.) than No. 8 (30 per cent.). In this respect the inheritance of the tumor rate may be compared to the inheritance of color, in which also in many cases until a complete analysis of the various factors has been made the color of the hybrids cannot be predicted.

7. There are certain other peculiarities observed in the case of strains with different tumor rates. Thus the cream which have the lowest tumor rate are mice which grow and mature more slowly than any other strain of mice, although they reach ultimately a large size, and they are very poor breeders. The hybrids between No. 10 and European which have the highest tumor rate grow rapidly and are very good breeders. The English strain also grows well and breeds well. But the parallelism between these characteristics and the incidence of tumors in the various strains is not apparent in every case. Thus certain strains grow very well and are good breeders without having a high tumor rate.

8. Mice belonging to strains with a low tumor rate did not develop tumors either by living in boxes in which mice with spontaneous tumors had lived a long time, although the boxes had been kept unaltered (not cleaned) during the course of the experiment or by living in the same box with mice spontaneously affected with cancer.

This agrees with the previous observations of Loeb, who under similar conditions, in which however rats with inoculated instead of spontaneous tumors were used, never observed a transmission of a sarcoma to another rat. It is however noteworthy that in strain No. 8 the large majority of cancers appeared in groups, inasmuch as several, in one case even as much as five mice, which were kept in the same box were simultaneously affected by cancer. Whether we have in this case which was not duplicated in the case of other strains to deal with an accidental occurrence we are unable to state at present.

9. From our investigations we may conclude that hereditary factors play a great part in the incidence of cancer among mice and that hereditary transmission is to a great extent responsible for the so-called endemic occurrence of cancer among animals. Certain observations especially of Borrel and Fiebiger concerning the occurrence of parasitic worms in certain kinds of cancer of animals indicate that also other factors of an infectious character may be responsible for this endemic occurrence.[1]

Miss Maud Slye, of Chicago, in experiments carried on simultaneously with our own, also came to the conclusion that the incidence of cancer varies in the different strains of mice which she had under observation according to a preliminary communication she made at the last meeting of the American Association for Cancer Research in May, 1913. In the discussion to Miss Slye's paper we mentioned some of the results of our work.

22 (839)

The influence of pregnancies on the incidence of cancer in mice.

By A. E. C. LATHROP and LEO LOEB.

[*From the Department of Pathology, Barnard Free Skin and Cancer Hospital.*]

In order to analyze still further the various factors causing the spontaneous development of cancer of the breast in mice we

[1] On a previous occasion (*Centralblatt f. allg. Pathologie*, Bd. XII, N. 22, 1911, p. 994) we published already a tree of one of the families of mice under our observation in which the hereditary transmission of tumor had been apparent. Cf. also *Interstate Medical Journal*, Vol. XX, No. 5, 1913.

undertook investigations into the influence of pregnancy in various strains of mice.

Accordingly we kept a number of mice from various strains after they were weaned separated from males throughout their life. Other mice from the same strains were allowed to breed in the usual manner. A few months after we had begun our experiments we received a recent communication by Bashford, who stated that he did not notice any influence of pregnancies on the incidence of cancer in mice.

Our results are as follows:

I. *English Strain.*—Never bred mice.

Group *A* (24 non-breeding mice): 46 per cent. have tumors. The corresponding percentage in bred English mice varies in different groups between 62 per cent. and 75 per cent.

91 per cent. of the tumors in never-bred mice occurred in mice older than 12 months, 36 per cent. in mice above 17 months of age, while in breeding English mice the corresponding percentages were 32 per cent. and 4.6 per cent respectively..

Group *B* (58 non-breeding mice): Experiment not yet finished. 17 per cent. of the mice have had tumors so far, almost all of them between 10 and 12 months of age. All others, 22 of which are already more than 12 months old, have not yet had tumors In control mice 68 per cent. had tumors at or under 12 months of age. We find therefore that even in English mice that are prevented from breeding a considerable number of tumors occur, but that the number of mice affected by cancer is distinctly decreased and that the age at which the tumors develop is higher in non-breeders than in breeders.

II. No. 8 (136 non-breeding mice). In 3.6 per cent. of the non-breeders the development of tumors was observed, in 100 per cent. of these above the age of 12 months, in 80 per cent. above the age of 20 months.

In breeding mice of the same strain 30 per cent. had tumors, 72 per cent. above 12 months of age and 36 per cent above 17 months. We find here a great reduction in the incidence of cancer and an increase in the age at which the tumors develop in non-breeding mice.

III. No. 8½ (48 non-breeding mice). In 10 per cent. tumors

developed, in 100 per cent. of these above 12 months and in 60 per cent. above 17 months, while in breeding mice of the same strain, the tumor incidence was 14 per cent., in 69 per cent. of which the tumors occurred above 12, in 8 per cent. above 17 months of age. There is here only a slight reduction in the cancer rate in non-breeding mice and a more marked increase in the age at which cancer occurred in non-breeding mice as compared to breeding mice of this strain.

IV. Carter (44 non-breeding mice): 4½ per cent. have tumors, 100 per cent. above 20 months of age as compared to 35 per cent. tumor incidence in breeding mice, in 63 per cent. of which the tumor developed at 12 months of age or below while in none it appeared in a mouse older than 17 months. Here we find therefore again a very marked difference in the same direction as in the previous strains.

Three further experiments the details of which do not need to be given here confirm these results. We may therefore conclude:

1. In breeding mice cancer of the breast occurs in a considerably larger number of individuals than in non-breeding mice.

2. In non-breeding mice the age at which cancer occurs is much higher than in breeding mice, and

3. The number of mammary cancers which occur in non-breeding mice varies in different strains. They are relatively more frequent in non-breeding mice from such strains in which in breeding mice the tumor incidence is greater and is relatively less frequent in non-breeding mice from strains in which also in breeding mice the tumor incidence is less.

4. In our preceding communication we stated that in some strains which are naturally poor breeders (the "creams"), the incidence of cancer is relatively low; while this factor may contribute to the low rate of tumors in such strains, it is in itself not sufficient to explain all or even the major part of the hereditary difference in the incidence in cancer in the various strains of mice.

ABSTRACTS OF THE COMMUNICATIONS, PACIFIC COAST BRANCH.

Fourth meeting.

San Francisco, California, October 9, 1913.

23 (840)

Skin reaction in streptothrix infections.

By EDITH J. CLAYPOLE (by invitation).

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California.*]

There are a number of cases of obscure infection, especially of the lungs, which pass frequently as tuberculosis, clinically showing many similar symptoms, without, however, tubercle bacilli. Quite a proportion of these cases are due to a streptothrix infection. Detection by sputum examination is not nearly so easy or so definite as in tuberculosis, and after working over the subject in a variety of ways it seemed possible that a skin test similar to that made by old tuberculin might help in the problem. From two species of these organisms, *S. eppingeri*, a partly acid-fast type, and *S. hominis* (III, Foulerton), a preparation was made exactly analogous to Koch's old tuberculin (a glycerine bouillon filtrate concentrated to 1/10th its original volume, making a 50 per cent. glycerin extract). The organisms were grown 4–6 weeks at 37° C. in 5 per cent. glycerine bouillon.

After making three slight abrasions of the skin on the upper arm with a von Pirquet chisel, the three preparations were gently rubbed in, leaving them to dry for 10 minutes. The tuberculin was used as a control in each case, and the streptotricin preparations compared with it.

Eleven cases of tuberculosis, six non-tuberculous lung infections, and five infants from 3½ to 17 months were tested with the following results:

11 Tuberculous.....	Old Tuberculin	Positive 8	Negative 3
	Streptotricin	Positive 1	Negative 10
6 Non-tuberculous.....	Old Tuberculin	Positive 1	Negative 5
	Streptotricin	Positive 6	Negative 0
5 Infants.....	Old Tuberculin	Positive 1	Negative 4
	Streptotricin	Positive 0	Negative 5

Streptothrix organisms in the form of the typical pinhead-sized granules were found in the sputum of all six cases reacting to streptotricin. Two cases showed a positive reaction to both tests, and in the sputum of one, both organisms were present in profusion; in the other, only the streptothrix. All these cases reacting to the streptotricin show the characteristic organism in good sputum specimens.

The reaction in the cases so far observed is a bright red hyperemic area from 7–10 mm. in diameter, a slight induration in the center, but without any tendency to form the dense induration of some of the tuberculin reactions. Examinations were recorded 24 and 36 to 48 hours after vaccination, but later in some cases where there was quite a tendency to induration, lasting even a week. Observations are now being made on other cases and on presumably normal people to determine the range and reliability of the reaction.

24 (841)

Further note on the influence of cholesterol on the growth of tumors.

By THEO. C. BURNETT (by invitation).

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

In previous papers it has been shown by Robertson and the writer, that cholesterol accelerates the growth of carcinoma in rats, and also, in view of the recent work of Wacker and of Ellis and Gardner, it may be a factor in the incidence of cancer.[1] In our original experiments the cholesterol was injected into, or around the tumor, and the criticism has been made, and justly too, we think, that the increase in the growth of the tumor might be due to the mechanical irritation of the injections, although we had previously controlled this possibility by injections of a balanced salt solution. We determined to test this matter further by making the injections on the opposite side of the body to that of the inoculations, and it has been the privilege of the writer to carry on these experiments in the absence of Robertson on sab-

[1] Robertson and Burnett, *Jour. Exp. Med.*, Vol. 17, No. 3, 1913, p. 344; PROC. SOC. EXP. BIOL. AND MED., Vol. 10, 1913, p. 140.

batical leave. Two sets of experiments were made, and may be briefly described.

Experiment 1.—Seventeen rats that had proved refractory to inoculation in April last (1913) were injected with 1 c.c. of a 2 per cent. emulsion of cholesterol in sodium oleate, on August 4 and 6, 1913. The injection was made on the left side. On August 6 they were all inoculated on the right side with the eighth generation of a Flexner-Jobling tumor. The injections of cholesterol emulsion were continued every alternate day thereafter, on the left side. On August 27, twenty-one days after inoculation, eight rats had developed tumors, ranging from 5×5 mm. to 13×15 mm. in size, the average for the eight being 9.7 mm. Bearing in mind that rats previously refractory may later become susceptible, and that tumors vary in virulence, this is not conclusive. The mistake was in not setting aside some of this lot as controls.

Experiment 2.—Forty "Chicago" rats were divided into two lots of twenty each. Lot *A* were injected with 1 c.c. of a 2 per cent. cholesterol emulsion on the left side, on August 11 and 13, 1913. August 13 both lot *A* and lot *B* were inoculated on the right side with portions of the same Flexner-Jobling tumor, eighth generation. It may be said in passing that this is the same strain of tumor used by us in our original experiments on the "Chicago" rats.[1] Lot *A* was then injected with 1 c.c. cholesterol emulsion every alternate day until September 3, 1913. Lot *B* were left untreated as controls. On September 3, twenty-one days after inoculation, thirteen rats in lot *A* had developed tumors ranging in size from 5×5 mm. to 25×10 mm., the average for the thirteen being 13 mm. Seven rats in lot *B* (controls) developed tumors ranging from 3×3 mm. to 20×11 mm. in size, the average for the seven being 9.5 mm. On September 15 the tumors were again measured, with the following result. Seven tumors in the "refractory" lot (one had retrogressed) gave an average diameter of 18.5 mm. Six tumors of lot *B* (one retrogressed) averaged 8.41 mm. Twelve tumors of lot *A* (one died) gave an average of 17.6 mm. The cholesterol-treated tumors have steadily increased in size, while the controls have just about held their own. Neither the "refractory" rats, nor those of lot *A* received any cholesterol after August 29.

[1] *Loc. cit.*

From the above results it is evident that our original conclusions are correct. Cholesterol has an accelerating action on malignant tumor growth, whether it be injected into the tumor, or carried to it by the circulation.

25 (842)

Nephritis in ground squirrels (Citellus Beechyi).

By WILLIAM OPHÜLS and GEORGE W. MCCOY.

[*From the Plague Laboratory of the U. S. Public Health Service at San Francisco and the Pathological Laboratory of the Stanford University Medical Department.*]

In the course of the examination of about 250,000 ground squirrels for plague 6 cases were noted in which there were gross lesions in the kidneys and which on microscopic examination presented evidence of chronic nephritis.

In one of these the lesions were very much like those in the experimental uranium nephritis of rabbits. There were large areas of cellular infiltration and fibrosis with atrophy of the tubules. The capsules of the glomeruli in these areas were slightly thickened. Some of the glomeruli showed a marked cystic dilatation. Other parts of these kidneys were practically normal except for a partial necrosis of the epithelium.

Two other specimens resembled closely the type of spontaneous nephritis in wild rats described by us in the *Journal of Medical Research* (1912, XXVI, 249). There was the same granular degeneration, necrosis and desquamation of the epithelium in some places with marked regenerative proliferation of the epithelium in others. There was the same tendency to the formation of epithelial cysts. The glomeruli showed some enlargement and proliferation of the capsular epithelium and a slight fibrous thickening of the capsule itself. In the interstitial tissue we found irregular areas of cellular infiltration and more or less fibrosis.

The three remaining cases were the most interesting ones in that they showed an entirely different type of the disease associated with the accumulation in many, usually somewhat dilated, tubules

of colorless crystalline masses which seem to consist of thick rhomboidal plates closely joined together in the form of rosettes. Sometimes they are so tightly packed as to form solid spherical bodies. One of us (Ophüls) has observed similar deposits in a human kidney in a case in which the renal pelvis was filled with large stones, apparently composed largely of urates. It seems most likely that these deposits are also deposits of urates. No evidence of stone formation in the pelvis was however present in the squirrels. The histological lesions in the squirrels are also similar to those in the human case mentioned. They involve the interstitial tissue very largely and have caused, in two cases especially, a very extensive development of cellular connective tissue with much destruction of kidney substance. The glomeruli in these areas show a slight fibrous thickening of their capsules. The rest of the tubules were normal except in one case in which there was some degeneration and evidences of proliferation in the tubules, similar to that found in the second type which resembles rat nephritis.

It is hardly necessary to say that arterial lesions were carefully searched for but there was no evidence of them in any of the specimens.

26 (843)

Experimental embolism of the arterioles in guinea pigs with hardened erythrocytes of Triton (Diemy Aylus) torosus and of Chondrotus tenebrosus.

By W. OPHÜLS.

[*From the Laboratory of Pathology of the Medical School of Leland Stanford Junior University, San Francisco.*]

The experiments were suggested by a desire to determine experimentally the changes, if there are any, which follow the complete obstruction of the vasa afferentia of the glomeruli of the kidney in mammals. A blocking of such small vessels may be accomplished by the use of the large erythrocytes of newts or salamanders which may be conveniently hardened in Orth's fluid, then washed in water, suspended in sterile salt solution and injected

by means of a hypodermic syringe directly into the left ventricle. The corpuscles after hardening measure about 32 × 12 micromm.

It is necessary to inject a rather heavy suspension of the corpuscles, otherwise so few glomeruli become obstructed that it is difficult to find them in sections; on the other hand, as most of the corpuscles are carried into the brain, convulsions and death are apt to occur if too many are introduced into the arterial circulation.

In the spleen the corpuscles lodge in the central arterioles of the Malpighian bodies and do not cause any lesions.

I have never been able to find the corpuscles in liver or lungs in spite of the study of many serial sections.

In the kidneys also they are not very numerous as a rule, the careful study of serial sections being required to find them. They block the vas afferens completely, but in spite of this the glomerulus remains absolutely intact and no histologic lesions follow elsewhere in the renal tissues. This would make it very improbable that the collapse and fibrosis of the glomeruli in arteriosclerosis is due directly to the mechanical obstruction of the vasa afferentia by the arteriosclerotic process.

In the brain the corpuscles are much more numerous both in the cortex and basilar ganglia. Curiously enough in brain sections of an animal which was examined a month after the injection the foreign corpuscles appear to lie outside of the blood-vessels in the perivascular lymph spaces which would suggest a degree of permeability of these vessels hitherto unsuspected. Apart from doubtful degenerative processes in some of the cells, there were no lesions in the central nervous system either.

If it should be of any physiological importance, the method might be used to determine by a proper selection of corpuscles the exact size of the arterial capillaries while fully distended under normal blood pressure.

27 (844)

A further note on specific hyperleucocytosis in immunized animals.

By FREDERICK P. GAY and EDITH J. CLAYPOLE.

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California.*]

In a previous communication,[1] we have mentioned the specific and extreme hyperleucocytosis which occurs on injecting a culture of the typhoid bacillus in a typhoid immunized rabbit. Further study shows that this reaction precedes and is remarkably more intense than the reaction that occurs on injecting *B. typhosus* in normal rabbits. In both instances there is an initial leucopenia two hours after injection followed by a rise and subsequent fall and a second higher rise in both normal and immunized rabbits. A mean of a considerable number of determinations shows that in immunized animals the first rise occurs at about 12 hours and averages a leucocyte count of 62,800. Counts of 100,000 to 150,000 have occurred not infrequently. This is followed by a fall at about 16 hours followed by a second rise which reaches at 18 hours an average of 74,700 leucocytes. In the normal animal these two rises are also evident, occurring at 18 and 26 hours respectively, and giving an average of 39,200 and 37,000 leucocytes to the cubic millimeter.

This specific type of leucocytosis has also been found to occur in rabbits immunized against red blood cells (sheep and guinea pig) and also against horse serum. In both these instances the highest leucocyte count occurs much sooner than in the case of typhoid immunized animals, somewhere between 4 and 8 hours following injection. It does not occur with equal intensity in all animals, and the intensity would seem, from preliminary observations, to bear some relation to the hemolytic titer or the precipitin titer of the animal concerned. This latter fact led to a crucial experiment designed to expose the mechanism by which the specific hyperleucocytosis is produced.

It is found that when a normal rabbit is given an intravenous injection of 1 c.c. of well-sentitized sheep-blood corpuscles, subse-

[1] *Journal American Medical Association*, LX, 1913, 1950.

quently washed to remove excess of hemolytic serum, that a hyperleucocytosis follows equalling in intensity the one produced in an immunized animal. No such phenomenon occurs in a control rabbit given sheep blood treated with the serum of a normal rabbit. In other words, the marked leucocytic response in the immunized animals is due to the tropic action of its serum on the antigen in question.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Fifty-sixth meeting.

College of Physicians and Surgeons, December 17, 1913.
President Ewing in the chair.

28 (845)

The influence of epinephrin on carbohydrate metabolism.

By Graham Lusk.

[*From the Physiological Laboratory of the Cornell University Medical College, New York City.*]

It has been stated that epinephrin stimulates the activity of the thyroid and thereby increases protein metabolism; that it inhibits the activity of the pancreas thereby reducing the internal secretion of that gland with resulting diabetes (school of von Noorden). Hari, working with curarized dogs, finds a higher respiratory quotient after administering epinephrin than before the dose had been given. Wilenko, using rabbits under the influence of urethan, finds that epinephrin injections are without influence on the respiratory quotients, and that when carbohydrate is administered at the same time, the respiratory quotient indicates that the combustion of sugar is largely suppressed. Falta has lately administered epinephrin to human beings and has noted a rise in the respiratory quotient.

In the experiments now reported it was found that in the case of a dog which received 50 grams of glucose per os and, at the same time, received 1 milligram of epinephrin per kilogram subcutaneously, the respiratory quotient rose to unity, remained at that level during five hours in spite of the fact that during this period about 10 grams of glucose were eliminated in the urine. The protein metabolism was unaffected. Therefore, adrenalin does not inhibit the pancreas causing diminished carbohydrate oxidation,

nor does it stimulate the thyroid causing increased protein metabolism.

29 (846)

An experimental study of heredity in bovine tuberculosis.

By HARLOW BROOKS, M.D.

[*From the Department of Medicine, University and Bellevue Hospital Medical College.*]

This study was conducted on what is probably the most valuable herd of Holstein Fresian cattle in the world. It has now extended over about ten years, hence several successive generations of stock have been studied under precisely similar conditions. The experiments have been supervised and verified by officers from the U. S. Department of Agriculture and the data are matters of official record in the register published by the Holstein Fresian Association. I am permitted to summarize and publish the results of the experiments by Mr. J. W. Dimick, the proprietor of Woodcrest Farm, where the problem is under study.

The number of animals comprised in the study is 425. The animals were originally selected because of their desirability from the standpoint of breeders and milkers or because of their "type" and entirely independent of their being or not being tuberculous.

The tuberculous animals greatly outnumbered the non-tuberculous and in most instances several generations of tuberculosis on both sides is known to have existed. 300 tuberculous animals were studied. The existence of tuberculosis was determined by the administration of treble the official dose of tuberculin, repeated in non-reacting animals three times at intervals of six months. All animals reacting to either test were removed at once to the tuberculous farm, the administration of which is entirely separate from that of the non-reacting herd. Little or no possibility of the transmission of infection from the tuberculous group to the healthy one exists.

With few exceptions tuberculous cows are bred to tuberculous bulls, the selection in any case is made for purposes of "type" and no account of the infection is taken in so far as breeding is concerned.

At birth the calves are immediately taken from the mother and no possible communication thenceforth exists between them except that the calf is given by bottle one feeding of the first milk secretion of the mother, removed by stripping and fed unsterilized. All subsequent feedings are made from a modified milk formula, based on pasteurized milk collected indiscriminately from sound and tuberculous animals. The feedings are made directly after pasteurization and before the milk has been cooled; pasteurization is begun immediately after the milk has been collected and before it has lost its natural heat.

Over 200 calves have been born of the tuberculous herd, not one has become tuberculous although all have been tested three times by massive doses of tuberculin. Some evidence exists tending to indicate that these animals are rather more resistant to tuberculosis than animals born of non-tuberculous parents.

No falling off in type, in milk production or fertility is present in these calves, no increased death exists among them as compared with the offspring of healthy cattle. We may also add that no falling off in value takes place and among calves of this ancestry are several of the most valuable cows and bulls in the world. These facts remain constant even where at least three generations of known tuberculous parentage exists.

As to the condition of the tuberculous animals themselves. None showing gross evidence of the disease are kept but in so far as the other reactors are concerned, practically all the world records as to fertility, milk and cream production are held by animals either themselves thus infected or who are the offspring of tuberculous ancestry.

The world's record of milk production was accomplished by a 7-year-old cow of this tuberculous group (Woodcrest Meta Vernon), herself also the descendant of three known tubercular generations. This cow gave in 365 consecutive days a total of 28,436 pounds of milk and during her five years of fecundity has given birth to five high-grade and perfect calves. A 23-month heifer of this group, sired and dammed by tuberculous animals, has beaten the world's record of milk production for this age by 4,000 pounds.

The object of the experiment is to produce a herd of Holstein

cattle entirely free from tuberculous taint and yet endowed with all the most valuable strain characteristics possessed by this breed of stock.

30 (847)

The function of the otic labyrinth in turtles.

By J. GORDON WILSON and F. H. PIKE.

[*From the Department of Physiology, College of Physicians and Surgeons.*]

The peculiar method of progression in serpents[1] and the widely different modes of progression in lizards, snakes and turtles[2] have attracted attention to the relation of the semi-circular canals to the processes of progression and maintenance of equilibrium in these forms.

The general results of labyrinthine extirpation in all these forms are similar to the results observed in other vertebrate types. There is, in the turtle, torsion of the head to the injured side, permanent deviation of the eyes and a tendency to crawl or swim toward the injured side, when the lesion is unilateral. The body on the uninjured side may be raised higher than on the injured side.

After bilateral operation, there are coarse wide tremors of the head which seriously interfere with grasping food. The gait on land is not markedly affected permanently, and there is no permanent torsion of the head to either side. The head may, however, be displaced directly upward and backwards in the first few days following extirpation. Swimming is a matter of great difficulty. When the turtle moves slowly, progress is fairly good, but agitation or hurry upset coördination and extreme disorientation results. The animal's reactions are not biologically adequate (Edinger).

It may be shown in turtles and snakes particularly that the otic labyrinth is a great proprioceptive organ for the head segment (Sherrington). The rôle of the labyrinth in the maintenance of equilibrium rests primarily upon its relation to the head, and only

[1] Henri, *Comptes rendus de la Soc. de Biol.*, Paris, 1899, I, IIe serie, 94–5.

[2] Trendelenburg and Kühn, *Archiv für Physiologie*, 1908, pp. 160.

secondarily upon its relation to the body. It may be shown that under certain conditions, the head is the only part affected.

31 (848)

The respiratory and cardiac variations of intrathoracic pressure and their significance in cardiac contraction.

By CARL J. WIGGERS.

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

When intrathoracic pressure is recorded by a trocar connecting with a calibrated Frank's segment capsule it is found that the intrathoracic pressure does not change smoothly with inspiration and expiration, but each respiratory variation consists of a series of negative and positive cardiac changes. The ratio between the cardiac and the respiratory variations range from 1 : 3 to 1 : 6. Thus, in an animal whose entire intrathoracic variation was 36 mm. of water, the cardiopneumatic changes were equal to 11 mm. during an apnea period, increased to 15 mm. in inspiration and fell to 9 mm. during expiration. A comparison with simultaneous intraventricular pressure curves shows that the negative pressure decreases slightly during the period of rising intraventricular tension; then, as the ejection period begins, gives a sharp vibration and then drops sharply until it reaches a turning point, after which the curve follows the reverse of the contour of the intraventricular pressure curve.

Are these variations in whole or in part responsible for the inspiratory fall of arterial and intraventricular pressures that occur when cardiac rhythm is regular? It is conceivable that the more negative pressure during inspiration might do this either by directly counteracting the cardiac systole or by diminishing its vigor through a decrease in the initial intraventricular tension at the beginning of systole. In either case the steepness of the isometric rise of the curve should show a decrease. That this is so is shown in experiments where considerable negative pressure is applied to the heart by a cardiometer over the top of which the pericardium was tied.

When extracardial pressures equal to those previously existing in the closed thorax are applied, however, the records so far obtained show no alteration in the steepness of the curve, nor is any difference discernible when the cardiometer is left open to the air or in communication with a tambour within which a pressure equal to 15 mm. of water develops during systole. Furthermore, the records taken from naturally breathing animals *by a sound correctly placed within the ventricular cavity* also show no decreased incline of the isometric rise; in fact, some records reveal a slightly steeper curve during inspiration.

These results indicate that such negative pressures as are normally developed within the chest during cardiac systole or the acts of respiration are without direct effect on intraventricular pressure and hence cannot be responsible for the fall of arterial pressures during *inspiration.*

32 (849)

The effect of gentian violet on enzymes, toxines and ultra-microscopic infections.

By JOHN W. CHURCHMAN.

[*From the Laboratory of Surgery, Yale University.*]

Since the observations reported by me, two years ago, on the effect of gentian violet on bacteria, studies have been carried on to extend these observations into the field of enzymes, toxines and ultra-microscopic infections. The original purpose of these experiments was to offer a new method of studying ultra-microscopic infections and to see if it might not be possible, by adding a dye to an infectious agent, to stain and thus to kill organisms too small to be seen. Experiments of a similar nature are now under way in this laboratory with inoculable tumors.

The following groups of active agents have thus far been studied:

1. Organized ferments (yeast). Yeast cells when stained with gentian violet lose entirely their power of fermenting sugar.

2. Unorganized ferments. (*a*) Ptyalin (salivary diastase). This ferment when stained with gentian violet is quite as active as

the unstained controls. (*b*) Pepsin. The activity of this enzyme was estimated by its action on wedges of egg albumen. It was found to be unaffected by the dye. (*c*) Trypsin. The dye was also without effect on this enzyme. (*d*) Rennin. The power of the enzyme to clot milk was entirely uninfluenced, even by prolonged and deep staining with gentian violet. (*e*) Thrombin. With this enzyme (kindly furnished by Prof. Howell) the results though not quite so convincing as with (*a*), (*b*), (*c*) and (*d*), were in general similar. Gentian violet staining does not destroy the power of the enzyme to clot blood plasma, though if present in large quantities the dye may *hinder* clotting somewhat.

In a word the organized ferment (yeast) is "killed" by staining with gentian violet; the unorganized ferments are unaffected.

3. Toxines. The toxines of tetanus and diphtheria were studied. With the former, the results were definite and constant; staining of the diphtheria toxine in no way impairs its toxicity. With the latter, the results were less constant; possibly some delay in the death of the experimental animals is produced by previously staining the tetanus toxine with gentian violet.

4. Ultra-microscopic infections. (*a*) Vaccinia. Rabbits were vaccinated on the back with stained and unstained vaccine. The potency of the vaccinia was unimpaired by staining, for equally good takes were obtained with both specimens. (*b*) Rabies. Experiments with this agent were kindly done for me by Dr. Williams at the N. Y. Board of Health Laboratory. Only a few experiments were done, but the dye was apparently without effect on the virus. (*c*) Anterior poliomyelitis. Seventeen *Macacus rhesus* monkeys have received injections of the virus into the sciatic nerve. The early experiments suggested very strongly that the virus of this disease was weakened or even entirely robbed of its virulence by staining with gentian violet. In one series, for example, the control animal died of typical anterior poliomyelitis in 7 days; the animal which received stained virus is still alive and well, 8 months after injection. In the later experiments inconstancy of the controls made positive deductions impossible; and some of the animals which received the stained virus developed the disease.

33 (850)

The preparation of soy bean urease in solid form and its use in urea determination.

By DONALD D. VAN SLYKE and GLENN E. CULLEN.

[*From the Rockefeller Institute for Medical Research, New York.*]

In the course of work in which we have been utilizing the soy bean urease, recently introduced by Marshall into analytical chemistry, we have found it advantageous to prepare and keep the enzyme in solid form. The fine-ground beans are covered with 5 parts of water and allowed to stand an hour with occasional shaking. The extract is then pressed through cheese cloth, and either filtered or centrifuged. The enzyme in solid form is obtained from the extract by either: (1) Precipitation, by pouring the extract into at least 10 volumes of acetone; (2) concentration of the extract to dryness at room temperature at a pressure less than 1 mm. The dry powder obtained by either method can be dissolved in a few seconds in 10 parts of water, and the solution obtained is so active that it permits very rapid analyses. *Urine* (human) is diluted tenfold. Three c.c. (= 0.3 c.c. urine) are mixed in a 100 c.c. test tube with 2 c.c. of an 8 per cent. urease solution. A drop of caprylic alcohol (to prevent subsequent foaming) is added, and the mixture allowed to stand ten minutes at room temperature (18° or over), three minutes at 40°, or two minutes at 50°. The ammonia is then drawn off by ten minutes' aeration (Folin's method) into 20 c.c. of N/50HCl. The stoppers are placed in the tubes as soon as the urease is added, and the aeration run a half minute before opening the tube to add the alkali (4 grams solid K_2CO_3). *Blood:* 5 c.c. of freshly drawn blood are mixed with 1 c.c. of 5 per cent. potassium citrate, 1 c.c. of 8 per cent. urease, and 4 drops of caprylic alcohol. Remainder as with urines. If Folin's colorimetric method for determining the ammonia is used, 1 c.c. of blood suffices. *Aqueous tissue extracts* are brought to a volume of 0.5 to 1 c.c. per gram of tissue, and 5 c.c. portions are treated as described for blood, except that only 1 drop of caprylic alcohol is needed. In case the extracts have been acidified with acetic acid to coagulate proteins, 1 c.c. of 15 per

cent. K_2HPO_4 is added before the urease. *Time law of urease action:* The velocity of urea decomposition is expressed by the isotherm,

$$t = \frac{1}{e}\left(x + a \log \frac{b}{b + x}\right),$$

where t is the time of reaction, e the enzyme concentration, and x the concentration of ammonia formed. The derivation of the equation will be given later.

34 (851)

A method for the estimation of sugar in small quantities of blood.

By ROBERT C. LEWIS and STANLEY R. BENEDICT.

[*From the Chemical Research Laboratory, General Memorial Hospital, New York City.*]

The red color obtained by heating a dextrose solution with picric acid and sodium carbonate is employed as the basis of the present method for the determination of blood sugar, the reaction being so delicate that it is possible to determine the dextrose in as little as 0.5 c.c. of blood. Following is the method in detail as ordinarily used by us.

Two c.c. of blood are drawn from a vein through a hypodermic needle into an Ostwald pipette, a little potassium oxalate in the tip of the pipette preventing clotting. The blood is discharged immediately into a 25 c.c. volumetric flask containing 10 c.c. of N/100 acetic acid previously heated in a boiling water bath. The pipette is rinsed once with distilled water. The flask is replaced in the boiling water bath and shaken occasionally for five minutes. After cooling, 1 c.c. of 5 per cent. dialyzed iron (Merck) is added to precipitate any protein still in solution. Distilled water is added to the mark, the contents of the flask are filtered, and an aliquot of the clear filtrate (10 c.c. or 15 c.c.) is measured into a large Jena test tube (200 × 22 mm.) and evaporated to 1 c.c. or below (but not to dryness) over a direct flame, two glass beads being used to prevent bumping. Two c.c. of saturated picric acid solution and 3 c.c. of 20 per cent. sodium carbonate are added

and the tube is placed in a boiling water bath for ten minutes. The contents of the tube are then cooled and washed quantitatively into a 10 c.c. volumetric flask. After making up to the mark, the red solution is filtered into the colorimeter chamber and read at once against a standard freshly prepared by the same procedure from 1 c.c. of a solution containing 1 mg. of dextrose per c.c. The standard is usually set at 15 mm.

If less than 2 c.c. of blood is collected, the quantities of N/100 acetic acid and of dialyzed iron must be correspondingly decreased. With 1 c.c. of blood 20 c.c. of the clear filtrate are taken for dextrose determination; with 0.5 c.c. of blood the coagulum must be thoroughly washed and the entire filtrate and washings used for analysis. In the latter case the standard is made one half as strong as usual and set at 30 mm.[1]

The method suggested yields results closely approximating those obtained by the Allihn gravimetric method.

35 (852)

Electric currents in conductors with distributed capacity considered in relation to the propagation of the nerve impulse.

By ALBERT C. CREHORE and HORATIO B. WILLIAMS.

[*From the Department of Medicine of Cornell University and the Physiological Laboratory of Columbia University.*]

Nearly two centuries ago it was surmised that the nervous impulse might be of the nature of an electric current, but in the absence of definite proof the hypothesis was rejected, especially as objections were raised to it which seemed insuperable. It is difficult, if not altogether impossible, to reconcile all experimental results with the consequences of the molecular theory. If, however, we regard the nerve as an electrical conductor with distributed capacity, we are able to account for many of the fundamental experimental phenomena and also to predict the results of new experimental conditions. It has long been known that the speed of electricity on wires is less than the speed in free space

[1] The reading of 10 c.c. of solution at 30 mm. in a Duboscq colorimeter is quite possible if a piece of thick glass tubing 50 mm. long and 16 mm. inside diameter is placed in the colorimeter chamber.

and the formulæ for calculating these velocities are well understood. The rate of propagation of electricity in a conductor similar in form, size and material to a nerve fiber should be, according to these formulæ, of approximately the same order of magnitude as has been measured for the rate of the nervous impulse.

The enormous reduction of velocity (about ten million times) is chiefly attributable to the great ohmic resistance of the conductor coupled with the electrostatic capacity. As a result of measurements on the phrenic nerves of cats and calculations based on data of microscopic sections of nerves, we have been able to construct an artificial "nerve" of glass, paper, tinfoil and graphite, whose total resistance and capacity are of the same order of magnitude as those of the cat's nerve. On applying the break E.M.F. of an induction coil to this artificial nerve and leading off to a string galvanometer in the usual manner we have obtained typical diphasic curves almost identical with those obtained from cat nerves stimulated with the same current. Of greater significance is the fact that we have been able to predict a change in the form of the curves with change in the nature of the applied E.M.F. and to predetermine the character of the change. As an example we may mention that the action current of nerves stimulated by the make or break of a constant current is of totally different form when registered as a curve from the diphasic curves obtained by applying a momentary E.M.F.

It seems at present altogether probable that the phenomena of electrotonus, the effects of lowering of temperature, anesthetics and other well-known phenomena of nerve will be found on investigation to be compatible with the theory that nervous phenomena are essentially electrical in nature.

36 (853)

Blood platelets and blood clotting.

By T. F. ZUCKER (by invitation).

[*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.*]

That the formed elements of the blood play a part in normal coagulation has long been known. Both leucocytes and platelets have been said to yield substances which contribute to fibrin formation. Leucocytes alone, however, will not coagulate fibrinogen. Cramer and Pringle[1] have recently shown that oxalate plasma freed from platelets by filtering through clay filters does not clot on adding an amount of $CaCl_2$ which causes a similar centrifuged but unfiltered plasma to clot in a short time. That platelets disintegrate during coagulation, and that the addition of oxalate preserved them is well known. As early as 1881 Fano[2] appreciated the fact that centrifuging was not sufficient to remove all cellular elements, and therefore resorted to filtration through a clay filter.

Regarding the effect of other anticoagulants, it has been observed by Buerker[3] and by Deetjen[4] that the breaking down of platelets in shed blood as observed under the microscope was inhibited by all those substances which can be used in preventing coagulation. They mention oxalates, citrates, NaPO, Na_2HPO_{43}, salts of Mn, Fe and Ni, $MgSO_4$, Na_2CO_3, peroxides and hirudin. The fact that these substances all have platelet preservative properties, does not necessarily mean that their anticoagulant effect is due solely to the fact that platelets remain intact.

The experiments of Cramer and Pringle seem to show that oxalate is an anticoagulant because it preserves platelets. That this is more generally the mode of action of anticoagulants is shown by the following data. Citrate plasma gives exactly the same results as oxalate plasma, which has also been verified by

[1] *Quart. Journ. Exp. Physiol.*, 6, 1 (1913).
[2] *Arch. f. Physiol.*, 1881, p. 277.
[3] Buerker, *Pflug. Arch.*, 102, 36 (1904).
[4] Deetjen, *Zts. physiol. Chem.*, 63, 1 (1909).

others. Of greater interest than citrate are the anticoagulants which do not affect calcium. A plasma was obtained by drawing blood into an equal volume of a 1 per cent. solution of $MnCl_2$ in 0.9 per cent. NaCl. If this is treated with enough Na_2HPO_4 to precipitate the manganese, it coagulates quite readily. On centrifuging this plasma at a moderate speed to remove red cells and leucocytes (this also throws down some platelets), the coagulation is slightly deferred. By filtering through a clay filter a plasma is obtained which is not coagulated at all by Na_2HPO_4. Further evidence is furnished by making use of the fact that platelets on disintegrating yield a vasoconstrictor material.[1] The manganese chloride plasma when tested on artery rings gives no constriction, but on addition of Na_2HPO_4 it clots and gives marked constriction. This is the same result obtained with citrate and $CaCl_2$, and indicates clearly that the change taking place on adding Na_2HPO_4 is disintegration of platelets.

The observation of Buerker that $MgSO_4$ preserves platelets I have verified by centrifuging a magnesium sulphate plasma fractionally and staining the sediment with Wright's stain. The same was observed with NaCl and Na_2SO_4. It is, however, much more difficult to separate platelets from plasmas containing high concentration of salts (NaCl, $MgSO_4$, Na_2SO_4) than with oxalate citrate or fluoride, one reason being that on centrifuging the leucocytes and platelets do not form a well-marked zone, but largely sink into the red cell layer.

If a fresh salted plasma (I have used $MgSO_4$ and NaCl) is thoroughly centrifuged to remove the platelets as far as possible, this plasma when compared with the cell containing residue after dilution of both with normal saline, shows a much greater clotting time, or does not clot at all. The high concentration of salts does not permit the use of the test for vasoconstrictor material.

Hirudin also as tested by Buerker's or Deetjen's method preserves platelets. O'Connor[2] and others have found that hirudin prevents the liberation of vasoconstrictor material in shed blood. This indicates that hirudin prevents coagulation not only by an antithrombin action, but also by preserving platelets. Which of

[1] Zucker and Stewart, *Zentr. f. Physiol.*, 27, 85 (1913).

[2] *Arch. f. Exp. Path. and Pharmakol.*, 67, 195 (1912).

these plays the more important rôle in keeping blood in a fluid state cannot as yet be definitely decided. It seems to follow from the results stated above that if hirudin is used in sufficient quantity to prevent platelet disintegration entirely, the antithrombin action will not come into play at all.

Concerning the physico-chemical mechanism of platelet disintegration, we can as yet say nothing definite. Ca ions seem to be essential, and on that basis the effect of calcium precipitants (oxalates and fluorides) can be explained. Whether citrate decreases the ionization of Ca sufficiently to make the remaining Ca ions negligible has, I believe, never been determined quantitatively. Mn salts do not affect the calcium, but act directly on the platelets, and the possibility suggests itself that the action of citrates may be of the same kind. Gessard[1] reports data which show an antagonism between Ca and Mn ions, *i. e.*, within certain limits additional calcium may overcome the anticoagulant effect of Mn. Regarding the mode of action of hirudin and salts used in "salted plasmas," nothing at all can be said.

To decide the question whether the platelet preservatives antagonize the disturbing action of calcium on colloidal equilibrium of the lipoids of the cell membrane (*i. e.*, surface layer) as recently suggested by Clowes[2] and West for citrate, would require further experiments.

The suggestion of Buerker that clotting is the immediate consequence of platelet disintegration acquires new interest in the light of the experiments with clay filters. It will be noticed that the material to which the name prothrombin has been given closely corresponds in properties to intact platelets. In that case thrombin would be equivalent to disintegrated platelets; thrombokinase would be any substance ($CaCl_2$, tissue juices) which accelerates the disintegration of platelets.

From the above data, it appears clearly that the condition of platelets is of the greatest importance in coagulation. We cannot say that preserving the platelets intact is the only means of keeping blood in a fluid condition, but it does seem that in every case where blood remains uncoagulated, the platelets are

[1] *Compt. Rend.*, 153, 1241 (1911).

[2] PROC. SOC. EXP. BIOL. AND MED., XI, 6, 1913.

involved in some way. Experiments now in progress on the part which platelets play in the formation of peptone plasma are reserved for a separate communication. It may be stated here that after injection of peptone into a dog, the platelets are not destroyed (a statement often met with), although they disappear from circulating blood, and that in the blood there appears a substance similar in its actions to hirudin, which preserves platelets and prevents formation of fibrin (antithrombin).

37 (854)

The output of fecal bacteria as influenced by fasting and by high and low protein ingestion.

By **N. R. BLATHERWICK** and **P. B. HAWK.**

[*From the Laboratories of Physiological Chemistry of Jefferson Medical College and the University of Illinois.*]

By means of a seven-day fast the daily excretion of fecal bacterial nitrogen by a 76-kilogram man (E) was reduced from 1.571 gram to 0.101 gram, whereas the actual weight of the excreted bacterial substance was reduced from 14.336 grams to 0.920 gram per day. The percentage of the fecal nitrogen which was present as bacterial nitrogen was decreased from 55.82 per cent. to 32.39 per cent. as a result of the fast. The percentage of dry bacteria in dry feces was slightly increased.

The output of bacterial nitrogen and the output of bacterial substance were approximately the same on a low protein diet as during fasting. With the ingestion of a high protein diet these values underwent an immediate pronounced increase.

The percentage of the fecal nitrogen which was composed of bacterial nitrogen was about the same in the periods of low and high protein ingestion.

There was no definite relationship between the excretion of fecal bacteria and that of urinary indican.

The ingestion of 5.23 grams of nitrogen *after* the fast was followed by an excretion of fecal bacteria which was only 1/14 as great as when four times that amount of nitrogen was ingested *before* the fast.

38 (855)

Further light on the conjugation of Paramæcium.

By LORANDE LOSS WOODRUFF.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

On December 7, 1913, conjugation occurred in a mass culture started from my pedigreed race of *Paramœcium aurelia*[1] at the 4,102d generation, showing that this race is a conjugating race when the proper conditions for its consummation are realized.[2]

Variations in the tendency to conjugate which are exhibited by pure races and lines of *Paramœcium* have led Calkins to the view that herein lies the clue to the directly opposite results derived from his races and from mine.[3] "The race that I worked with in 1901 was a conjugating race which died out in the 742d generation. Woodruff's long line of over 3,500 generations is a non-conjugating race and the two races cannot be compared in regard to vitality, since normal conjugation was prevented in the conjugating race, whereas in the non-conjugating race there was no artificial prevention of a normal process."[4]

Since conjugation has now occurred in animals from my race there is no evidence extant that a "non-conjugating" race of *Paramœcium* exists.

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[1] The possibility of conjugation occurring in the main lines of the pedigreed race is prevented by daily isolation of the individuals. For details of this race cf. Woodruff: PROC. SOC. EXP. BIOL. AND MED., 1912, Vol. ix, p. 121.

[2] Details of this experiment will appear in the *Journ. Exp. Zoölogy*, Feb., 1914.

[3] G. N. Calkins (*J. Exper. Zoöl.*, Nov., 1913, p. 509): "The life history of conjugating lines has shown that if conjugation is prevented, the race dies out." L. L. Woodruff (*Archiv f. Protistenk.*, Jan., 1911, p. 266): "I believe this culture shows clearly that *Paramœcium aurelia*, when subjected to suitable culture conditions, has the power of unlimited reproduction by division without conjugation or artificial stimulation."

[4] Calkins, PROC. SOC. EXPER. BIOL. AND MED., Vol. 10, 1913, p. 67.

39 (856)

Effect of castration on weight of pituitary in rabbits.

By A. E. LIVINGSTON.

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

The object of the present investigation was to observe the effect of castration, in male and female rabbits, on the weight of the hypophysis. Some workers have recorded an increase in the weight of this gland after removal of the generative organs, *e. g.*, Fichera[1] ('05) found this to be the case in guinea pigs, rabbits, domestic fowls, cattle, and buffaloes, and Kon[2] ('08) has observed the same results in man. Marrassini[3] and Luciani ('11), on the other hand, also using guinea pigs, rabbits, sheep, cattle, and domestic fowls deny that this is the case. The question therefore at the present time appears to be an open one.

My observations have been made on two series of rabbits. The first series consisted of fifty animals including about an equal number of males and females. The sexes were separated and kept in adjoining pens, in the open air, under similar conditions as regards feeding and attendance. From approximately one half of each group the sexual glands were removed, the other half being kept as controls. The body weights were recorded weekly, on the forenoon of the same day (Saturday), before the animals had been fed.

They were killed at intervals varying from 26 to 208 days after castration, the body weight, minus the gastro-intestinal contents (the reduced body weight), was determined and the pituitary was removed with special care and weighed at once on a chemical balance. It was found that for the males the average weight of the pituitary, in milligrams per kilo of reduced body weight, was 11.57 mg. for the castrated and 10.23 mg. for the controls; while among the females the average pituitary weight,

[1] Fichera, *Archives ital. de Biol.*, Vol. 43, p. 405, 1905.

[2] Kon, *Ziegler's Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie*, Vol. 64, p. 233.

[3] Marrassini et Luciani, *Archives ital. de Biol.*, Vol. 56, p. 395, 1911.

for the spayed animals, was found to be 12.74 mg., and for the normals 13.49 mg.—a variation no larger than would probably be found among two groups of normal rabbits.

Several objections might be made to the above experiment: (1) the ages of the animals were uncertain, (2) it was not known whether the females had ever been pregnant, (3) the castrated and control animals were not necessarily of the same litter. For this reason another investigation was undertaken on a second series of rabbits.

In the second experiment most of the operated and control animals were selected from the same litter. It was known that, with a few exceptions, the females had never been pregnant. The animals were also younger than those of the first series, many weighing less than one kilo. The sexes were separated and kept under the same conditions as in the first experiment. At the end of about four months after operation a control and an operated animal were killed by coal gas on the same day, the reduced body weight recorded, and the pituitaries removed and weighed as before.

In the male group there were ten animals which could be controlled by eight of the same litter. The average weight of these pituitaries in milligrams per kilo of body weight is for the castrated 15.3 mg. and for the controls 15.8 mg., which shows a difference so small that it is entirely negligible. Among the females six were controlled by five of the same litter and in this case the average for the spayed animals is 16.49 mg. and in the controls 13.27 mg. or an increase of about 24 per cent. by this grouping.

It is significant to note that the curves of growth plotted from the weights taken each week show a distinct gain of the operated over the control animals in case of the males but not in case of the females, thus agreeing with Hatai's[1] ('13) results from the albino rat in that when castration is followed by an overgrowth in body weight there is no increase in the weight of the pituitary. The average for the whole series of twenty castrated males was found to be 15.94 mg., while the seventeen controls gave an average of 14.38 mg., thus showing a gain by the castrated males of less than

[1] Hatai, *Journal of Experimental Zoölogy*, Vol. 15, p. 297, 1913.

10 per cent. The whole series of females composed of ten spayed and eleven controls gave an average for the operated animals of 16.35 mg. and for the controls 13.71 mg. or a gain of 18 per cent. The curves of growth from this grouping are essentially the same as by the grouping just mentioned.

From these results it would seem that the apparent increase in weight of the pituitary after castration in case of the male rabbits should be entirely neglected, since it is no more than would be readily shown by two groups of normal animals. In case of the females of the second series the increase in weight of the pituitary of the spayed rabbits, although not marked, is quite distinct, and is accompanied by no response in overgrowth of body weight as is shown in case of the males.

40 (857)

Effect of thyroidectomy followed by thyroid feeding on weight of pituitary in rabbits.

By A. E. LIVINGSTON.

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

The object of this experiment was to determine the effect upon the weight of pituitary in the rabbit following the administration of sheep's thyroid. Both males and females were used. The male rabbits were divided into two groups. The animals of one were thyroidectomized and those of the other used as controls. To one half of the thyroidectomized animals and one half of the control group a capsule containing one tenth of a gram of Armour and Company's desiccated sheep's thyroid was administered on alternate days. The females were treated in precisely the same manner and thus each sex was composed of animals under four conditions: first those which were entirely normal, second those normal and fed thyroid, third those thyroidectomized and not fed thyroid, and fourth thyroidectomized and fed thyroid.

The males and females were kept separate but under the same conditions and each animal was weighed once a week. All the animals were killed at the end of about six months after operation

and in every case of thyroidectomy postmortem examination showed that the thyroid had been completely removed. The body weight of each animal was corrected by deducting the weight of contents of stomach, intestines, and bladder. The pituitary was carefully dissected out and weighed to the tenth of a milligram and the weight of each pituitary was then calculated in milligrams per kilo of corrected body weight.

The average weight of the pituitary, of the animals under the above-described conditions, in milligrams per kilo of corrected body weight may best be seen in the following table. The figures in parenthesis indicate the number of animals used in each case.

FEMALES.

Thyroidectomized.		Controls.	
Fed (4).	Not fed (2).	Fed (4).	Not fed (4).
14.72	14.80	14.56	13.90

MALES.

Thyroidectomized.		Controls.	
Fed (3).	Not fed (2).	Fed (4).	Not fed (4).
13.26	23.43	12.81	10.79

In the literature dealing with this subject it is generally agreed that removal of the thyroid is followed by an increase in the weight of the pituitary, but whether this increase is due to absence of the internal secretion of the thyroid or to some other cause has not been determined. If feeding thyroid to rabbits which have been thyroidectomized prevents the increase in size of the pituitary this would point to the conclusion that the determining factor is to be found in the internal secretion. Such seems to be the case in the male thyroidectomized group where no increase in pituitary weight is evident while thyroid substance is being supplied, but when not fed the weight of the gland is almost double the normal. The female thyroidectomized group however does not show this but here it will be observed that apparently the pituitary did not increase in size after removal of the thyroid where no desiccated gland had been fed and this might be explained by supposing that in these individual animals the glands happened to be unusually small to begin with.

No effect on the pituitary weight was observed from feeding desiccated thyroid to normal rabbits.

The substance fed was proved to be active by administering it

to rabbits employed in another experiment where, in somewhat larger doses, it rapidly produced toxic symptoms.

The number of animals used in this experiment is, of course, too small on which to base any definite statement, but in the thyroidectomized group of males it appears to point to the fact that thyroid feeding does prevent the enlargement of the pituitary which would otherwise follow on removal of the thyroid.

41 (858)

Method in the investigation of sensibility after the section of a cutaneous nerve. (Preliminary communication.)

By EDWIN G. BORING.

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

The difficulty of obtaining from clinical subjects, untrained in introspection, reliable accounts of the changes in cutaneous sensibility occurring during the regeneration of a cutaneous nerve has been partially obviated by Head[1] and by Trotter and Davies[2] through the use of the experimental method with themselves as subjects. These observers, however, did not take the precaution to make sure of their own ability to give the most accurate descriptions of cutaneous complexes, nor did they work with areas small enough to permit the application of the most exact experimental methods available. For this reason the writer has sought to conduct an experiment with such changes in procedure as should make for a more detailed and thorough description of the sensations involved. The writer acted as subject, and the conditions of the experiment were established by a section of the anterior branch of the internal cutaneous nerve. The following points may be noted:

1. A special attempt was made, during the year preceding the operation, to train the subject in the observation of cutaneous sensation and in the analysis of the sensational complexes mediated by the normal skin. Care was taken to distinguish between the

[1] *Brain*, 28, 1905, 99; 31, 1908, 323.

[2] *Jour. Physiol.*, 38, 1909, 134.

qualities of cutaneous contact, cutaneous pressure, and deep pressure, all of which can be distinguished by practice; between warmth, heat, and burning heat (the last two are complex); and to some extent between the different modes of cutaneous pain. Besides this special training, the subject has had the advantage of general introspective practice, obtained during several years of psychological investigation.

2. The final introspective practice series were so arranged as to supply objective norms for that area of the skin which was afterwards affected.

3. The selection of the nerve for section was such that the region affected was sufficiently small to permit of a more careful exploration than would have been possible if it had been necessary to cover a large area.

4. Except for purposes of comparison with other researches, areal stimuli were discarded as not sufficiently adapted to the investigation of the skin, in which sensibility is distributed in a punctiform manner. Emphasis was laid upon the isolation of the separate sensory spots, and an effort was made to determine, not only the strength of stimulus which would just arouse sensation in each one of these spots, but also the intensity of sensation aroused in each one by a constant strength of stimulus. For pressure, hairs were employed; and for pain, two needle algesimeters. Warmth and cold were studied by means of a hollow brass cylinder through which water of different temperatures was passed. The cylinder was drawn over the skin by a kinesimeter,[1] which controlled the rate of exploration and the pressure of the stimulus; a record of the position and of the intensity-value of the spots was taken simultaneously on a kymograph drum.

5. Orientation on the surface of the arm was obtained in most of the work by means of coördinate lines, impressed in a given relation to a set of tattoo marks by a rubber stamp. More accurate localization of the points was secured by placing the arm in a permanent plaster cast, to which was rigidly attached a vernier stage, carrying an indicator, that moved just over the surface of the skin. By reference to the indicator and the vernier scales, points could be identified to within less than one half of a millimeter.

[1] *Am. Jour. Psychol.*, 6, 1894, 424; 7, 1895, 150.

The section of the nerve was performed in January, 1913, and sensibility, at the time of writing, is not yet normal. It is, therefore, too early to present any definite conclusions. In general, however, the following points, subject to such modification in details as a subsequent working over of the data in the light of the completed experiment may necessitate, may be noted: (1) The section of the cutaneous nerve did not destroy the sensibility of the subcutaneous tissue, which provided to a considerable extent the capacity for localization. (2) With the exception of certain early general pains, that were probably, strictly speaking, not of cutaneous origin, it may be said that the return of warmth, cold, pressure, and pain began at approximately the same time. (3) The regions insensitive to these four qualities of sensations, both immediately after the section of the nerve and during the period of returning sensibility, were approximately, but by no means exactly, the same. (4) On the whole, the return of sensitivity tended to begin at the outside of the affected area and to progress toward the center, although decided irregularities in this course appeared. (5) In general, hypoesthesia preceded normal sensitivity, periods of hyperesthesia, however, being noted for pain and for cold. (6) The observations up to the present time indicate that the return to normal sensitivity will not be simultaneous in the cases of the four sense qualities and that the return in the case of temperature sensations is the more rapid.

42 (859)

The alleged discharge of the internal secretion of the pancreas into the lymph.

By **A. J. Carlson** and **F. M. Drennan.**

[*From the Hull Physiological Laboratory of the University of Chicago.*]

In 1898 Biedel[1] reported a "new form of experimental diabetes" by ligation of the thoracic duct and by establishing a fistula of the thoracic duct. These results were interpreted as proving that the internal secretion of the pancreas reaches the blood indirectly via the lymph of the thoracic duct, and led to attempts to modify or control diabetes by treatment with lymph from the thoracic

[1] Beidl, *Centralb. f. Physiol.*, XII, p. 624.

duct, with contradictory and practically negative results.[1] In cases where we have adequate tests for the internal secretion of an organ (for example, the adrenal glands) it has been shown that these secretions pass directly into the blood, not into the lymph. Despite this, the view that the internal secretions are discharged primarily into the lymph appears to be an attractive one to many physiologists, as shown by the survival of this theory in the case of the thyroids, and the recent attempts to secure evidence for the theory in the case of the hypophysis.

It is not clear that the glycosuria reported by Biedl following interference with the thoracic lymph is true pancreatic diabetes. Ligation of the thoracic duct may cause a temporary hyperglycosuria owing to injury of the liver by the edema from the back pressure of the lymph. And the fistula experiments do not exclude a temporary glycosuria due to the operation and the anesthesia.

We have repeated the thoracic duct fistula experiment of Biedl in two dogs with negative results. We found it impossible to maintain a continuous flow of lymph with a cannula in the duct owing to clotting. We therefore ligatured the veins in such a way that the thoracic lymph discharged into the external jugular vein, and this vein was slit open and secured to the skin, thus allowing free escape of the lymph. The dogs were under constant observation throughout the experiment, so that there was no retention of lymph from clotting at the slit in the jugular vein. In Dog I there was free escape of the thoracic lymph for 32 hours, in Dog II for 33 hours. The urine drawn from time to time by catheter showed no sugar at any time. The dogs showed an abnormal thirst, but the total secretion of urine was less than normal, owing probably to the continued loss of lymph.

Since extirpation of the pancreas brings on diabetes in from 6–10 hours, while complete elimination of the thoracic lymph for 32–33 hours does not induce even mild glycosuria, it is evident that the internal secretion of the pancreas enters the blood directly and not indirectly via the thoracic duct.

[1] The literature is discussed by Allen, "Glycosuria and Diabetes," Boston, 1913.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Fifty-seventh meeting.

Rockefeller Institute for Medical Research, February 18, 1914.
President Ewing in the chair.

43 (860)

Complete periodic nuclear reorganization without cell fusion in a pedigreed race of Paramaecium.

By **Lorande Loss Woodruff** and **Rh. Erdmann.**

[*From the Osborn Zoölogical Laboratory, Yale University.*]

This preliminary communication presents the main results derived from the cytological study of specimens preserved throughout the life of a pedigreed race of *Paramaecium aurelia*, as well as of a series of specimens preserved daily, during the past four months, from subcultures of this race carried under constant environmental conditions.

During the past six and three quarter years this pedigreed race has been carried with the following chief results:

1. More than 4,250 generations have been attained to date (February 18, 1914) without the fusion of individuals or the advent of periods of marked physiological depression, though periodically well-defined normal morphological changes have occurred.[1] This demonstrates that the so-called "life cycle" of *Paramaecium* is non-existent when proper environmental conditions are supplied.[2]

2. Minor periodic fluctuations in the division rate, termed rhythms, have been demonstrated, recovery from which is autonomous.[3]

[1] Woodruff, *Amer. Naturalist*, XLII, 1908, p. 526.

[2] Woodruff, *Biol. Bull.*, XVII, 1909, p. 287; Proc. Soc. Exp. Biol. and Med. IX, 1912, p. 121.

[3] Woodruff, *Archiv für Protistenkunde*, XXI, 1911, p. 263.

3. The rhythms have been shown to be independent of environmental changes and due to inherent phenomena in the cell.[1]

The present cytological study demonstrates that:

1. The rhythms in the division rate of *Paramaecium* are the physiological expression of profound nuclear changes.

2. These periodic nuclear phenomena involve the formation of a complete new nuclear apparatus by a definite sequence of morphological changes disintegration of old macronucleus; multiple division of micronuclei, formation of new macronuclear Anlagen which simulate typical conjugation. This results in the reorganization of the cell without the fusion of two animals.

This nuclear reorganization is evidently a normal substitute for typical conjugation in this race, but does not preclude its occurrence for the latter process has occurred in subcultures from this race subjected to environmental conditions suitable for its consummation.[2]

Details of this remarkable process, together with a discussion of its theoretical importance from the standpoint of the sexual potentiality of unicellular organisms and the physiological behavior of long pedigreed races of Infusoria, will be presented in another paper.

44 (861)

The aggressin-like action of anaphylatoxin.

By HANS ZINSSER and J. G. DWYER.

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*]

In the course of experiments upon the repeated injection of anaphylatoxin into guinea-pigs the writers noticed a phenomenon which suggested to them that the anaphylatoxic substances may possibly possess properties similar to those described by Bail for his "aggressins." In the earlier experiments the anaphylatoxins were prepared with typhoid bacilli by emulsifying one slant of the bacteria in 8 c.c. of fresh guinea pig complement and allowing

[1] Woodruff and Baitsell, *Jour. Exper. Zool.*, XI, 1911, p. 339. Erdmann, *Archiv. für Protistent.*, XXIX, 1913, p. 118.

[2] Woodruff, *Jour. Exper. Zool.*, XVI, 1914, p. 237.

the emulsion to remain in the incubator for 6 hours. At the end of this time centrifugation for one to two hours at high speed was used to throw down the bacteria.

Although the supernatant fluid after such centrifugation was clear, nevertheless it appeared in many cases that not all the bacteria were thrown down. Intraperitoneal injection of the anaphylatoxin occasionally resulted in the rapid death of the guinea-pigs with extensive proliferation of typhoid bacilli in the peritoneal exudate and presence of the organisms in the heart's blood.

Since the lethal dose of the typhoid culture employed varied between 1/19 and 1/50 of an agar slant (for guinea-pigs of about 200 grammes on intraperitoneal injection) and since the amounts of bacteria injected with 2 to 3 c.c. of an apparently clear anaphylatoxin could not have been anything like as much as the smallest lethal dose, special experiments were carried out to determine this point. An example of such experiments is the following:

EXPERIMENT 2, 9, 14.

Anaphylatoxin prepared in the ordinary way,—centrifugalized for over one hour until supernatant fluid appears absolutely clear. Cultivation experiments from this fluid show, however, that it is not absolutely sterile,—a few colonies resulting from a planting of several loopfuls.

	Dose Typhoid culture		Intraperitoneal injection	
Control				
No. 1	1/10	+	3 c.c. salt solution	= death in 52 hrs.
2	1/20	+	3 c.c. salt solution	= death in 18 hrs.
3	1/50	+	3 c.c. salt solution	= remains alive
Experiment				
1	1/100	+	3 c.c. anaphylatoxin	= death in 22 hrs.
2	1/100	+	3 c.c. anaphylatoxin	= death in 18 hrs.
3	1/200	+	3 c.c. anaphylatoxin	= death in 18 hrs.
4	1/200	+	3 c.c. anaphylatoxin	= death in 35 hrs.
5	1/200	+	3 c.c. anaphylatoxin	= death in 35 hrs.
6	1/300	+	3 c.c. anaphylatoxin	= remains alive

A number of similar experiments were made in all of which it was found that 1/200 to 1/300 of a typhoid culture combined with 2 to 3 c.c. of anaphylatoxin would bring about the rapid death of a guinea-pig by typhoid infection within a short time, when 1/50 of a culture alone, and in some cases as much as 1/20 and 1/10 of a culture alone, did not kill. 3 c.c. of anaphylatoxin

injected alone intraperitoneally either did not kill or whenever it did, the peritoneum was found filled with free typhoid bacilli. In some of the experiments the bacteria found in the peritoneal cavities of dead guinea-pigs appeared larger than organisms of the same strain taken from agar, having some of the characteristics of which Bail speaks as "Tierische bazillen." In one of our experiments, so far, they have shown resistance to agglutination.

We have carried out several experiments to determine whether or not this "aggressin" action is specific but owing to the difficulties of entirely ridding the anaphylatoxin of the bacteria with which it was produced we have been prevented from coming to definite conclusions. Filtration through Berkefeld candles in a few cases in which it was tried seemed to render the anaphylatoxin harmless when intravenously injected. From a number of experiments carried out with the *Staphylococcus aureus* and the *Bacillus prodigiosus*, however, we are inclined to believe that the action is not specific.

It seems not unlikely to us that the aggressins with which Bail worked were rather of the nature of "anaphylatoxin" and that the invasive properties of bacteria may well be gradually enhanced in the animal body as contact with the serum induces the formation of these substances. We abstain from further theoretical expansion of the ideas suggested by these experiments at present.

45 (862)

(a) The lipolytic activities of human duodenal contents. (b) The separation of the castor bean lipases.

By K. GEORGE FALK.

[*From the Harriman Research Laboratory, Roosevelt Hospital.*]

Some years ago, Loevenhart[1] showed that extracts of the pancreas and of the liver of various animals contained different lipases. The pancreas extracts exerted greater lipolytic action under comparable conditions toward complex esters such as glyceryl triacetate, and the liver extracts showed greater action toward simple esters such as ethyl butyrate.

[1] *J. Biol. Chem.*, 2, 429 (1907).

During the past year, a number of experiments were carried out on the lipolytic actions of duodenal contents of human beings, both after fasting and after taking food. The Einhorn or the Palefski tube was used to collect the secretions. The behavior was tested, with toluol as antiseptic, toward ethyl butyrate and triacetin under various conditions. The results obtained may be divided into two groups. Greater action toward ethyl butyrate than toward triacetin was observed when no food had been taken for at least twelve hours and the pancreatic juice therefore probably absent. Very much greater action toward triacetin than toward ethyl butyrate was observed in the cases in which food had been taken and the pancreatic juice was present. These relations were observed in a number of cases, but in several cases exceptional results were obtained. Under apparently normal conditions of digestion, when the activity should have been that of the pancreatic juice, that is, very large toward triacetin, a greater action toward ethyl butyrate was observed. Also, when pancreatic juice was expected to be absent, the activity observed in several cases was similar to that observed when it was present. Since, however, relatively few of these cases were observed, the greater activity toward triacetin of duodenal contents containing pancreatic juice, and the greater activity toward ethyl butyrate of contents without pancreatic juice, may for the present therefore be looked upon as the normal.

From the diagnostic point of view, these results indicate that the lipolytic activity, and perhaps also the activities of the other enzymes of duodenal contents, may sometimes be caused by secretions other than the pancreatic juice, even if the conditions for obtaining the latter are apparently favorable.

Since vegetable substances offer a more constant and satisfactory material for an extended experimental investigation, the lipase of the castor bean is being studied from various points of view. Two active preparations have been obtained from castor beans, which correspond to the two lipases just spoken of. One of these is more active toward ethyl butyrate than toward triacetin, the other more active toward triacetin than toward ethyl butyrate. In brief, the former may be obtained by extracting oil- and husk-free castor beans with water and precipitating with ammonium

sulphate or acetone, the latter by extracting the castor bean preparation with 1.5 normal sodium chloride solution and removing the salt by dialysis.

It is interesting to note that the different lipases found in extracts of animal organs, are present in the duodenal contents of human beings and may also be obtained from a vegetable substance such as castor beans.

46 (863)

Modifications of the Abel vividiffusion apparatus.

By **W. G. MACCALLUM, M.D.** and **R. A. LAMBERT, M.D.**

[*Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.*]

The apparatus for the study of the circulating blood by a process of diffusion devised by Drs. Abel, Rowntree and Turner has impressed us as such a brilliant stroke of inventive genius that we have hastened to imitate it and apply it. The scope of this method is perhaps not yet fully appreciated, but it has interested us particularly in affording a hopeful way of studying the inorganic constituents of the blood, especially in connection with tetany where we think that calcium plays a considerable rôle. The results of these experiments are as yet entirely incomplete but we wish to describe several modifications in the technique which may have been tried and discarded by Dr. Abel but which seem to us to be helpful.

The branched glass end pieces which allow the current of blood a choice of several paths are extremely difficult to make and are very fragile. Unless they are made very precisely, one path becomes easier than another in which blood may circulate slowly or stagnate. With this arrangement blood passes once only through the length of the apparatus and back again, but since the stream bed, with these many possible channels, is very wide, the current must be relatively slow.

We have constructed two or three machines in which the blood circulates back and forth eight or ten times before returning to the vein. In the first model the connections were made as in a

steam radiator with "U" tubes of glass set in discs of hard rubber fixed on a central glass rod. All this was enclosed in a water jacket. In spite of our fear that the blood pressure might be insufficient to force the blood through this long course (about 80 inches) it works well, and we have carried on an experiment for five hours. The difficulty in preventing leaks where the celloidin tube is tied on the glass we have obviated by first wrapping the joint with rubber adhesive plaster and then tying. The difficulty in tying on the tubes of the inner row when the "U" tubes are ranged round a disc, we have removed by spreading out the "U" tubes flat in a square frame made of rubber and glass which can be turned over when it is necessary to tie the lower row. When the sheaf of tubes is enclosed in a water cylinder plugged at both ends, it is impossible to remedy a leak except by stopping the experiment, but we have simply laid our square frame in an enamel pan of the fluid covered with a glass lid and can reach any part of it at any time without disturbing the circulation.

Hirudin is very expensive and we have therefore defibrinated the blood of our animals realizing the possible objections to this. Instead of starting with the celloidin tubes full of salt solution they are filled with defibrinated blood of another normal dog and enough of this is kept in a funnel or tank connected with the inlet tube to allow some blood from the machine to run through the outlet tube into the vein while a similar amount is bled from the carotid and defibrinated. This is poured into the funnel and the process repeated till the blood no longer clots when the inlet tube is connected with the carotid canula and circulation proceeds. So rapid is the torrent of blood through such a continuous channel that we have twice connected the inlet and outlet tubes directly with artery and vein without defibrinating and kept up the circulation for an hour without the formation of any clot. If the blood pressure sinks, through faulty etherization or other reason the blood clots, as happened in another experiment.

The most important and difficult part of the problem is the calculation of the proper fluid in which to immerse the apparatus. In our effort to abstract calcium we have tried several and are at present using a fluid calculated to correspond as closely as possible with Abderhalden's published analysis of the inorganic constituents of the dog's blood with the omission of the calcium.

With this apparatus dialysis, at least in so far as the inorganic substances are concerned, is rapid and satisfactory.

47 (864)

On the alleged rôle of hematin in the production of the malarial paroxysm.

By E. E. BUTTERFIELD and L. S. BENEDICT.

[*From the Pathological Department of Bellevue and Allied Hospitals.*]

The view has been advanced that the symptoms of the malarial paroxysm are due to the toxic action of hematin[1]. The basis for this view rests, (1) in the alleged identity of hematin and malarial pigment, and (2) in the effect produced by intravenous injections of hematin solutions in rabbits. Inasmuch as hematin has not yet been isolated from the fresh organs of an undoubted case of malaria, malarial pigment cannot be justly identified with hematin. The present communication deals chiefly with the temperature curve in rabbits after intravenous injection of from 0.0023 g. to 0.0370 g. of hematin per kilo body weight.

The hematin was prepared from hemin by the method of Piloty.[2] The hemin was prepared from ox blood by the Piloty modification[3] of the Schalfejeff method and recrystallized from pyridin. The hematin was dissolved in 0.9 per cent. NaCl solution containing 1.5 per cent. $NaHCO_3$. All solutions used contained 0.5 g. hematin in 100 c.c., were microscopically clear, and were sterile.

The rectal temperatures of the rabbits were taken every 30 minutes after injection, with a clinical thermometer which had been checked against a P. T. R. thermometer. Two minutes was allowed for the registration of the maximum temperature. Fresh rabbits were used for each series of experiments; no animals were reinoculated.

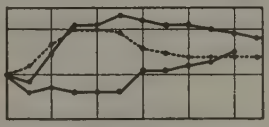
Twelve rabbits received hematin solution, eight rabbits received the solvent (0.9 per cent. NaCl solution containing 1.5 per cent.

[1] W. H. Brown, *Journal of Exp. Med.*, XV, p. 579, 1912.

[2] Piloty, *Ann. d. Chemie*, CCCLXXVII, p. 358, 1910.

[3] Piloty, *l. c.*, p. 344.

$NaHCO_3$) intravenously in corresponding volumes, and five rabbits were used as controls for accidental temperature fluctuations. The maximum rises in temperature for the individual hematin animals were, 3.5, 2.4, 1.8, 1.8, 1.7, 1.7, 1.5, 1.3, 1.2, 0.8, 0.7, and 0.6 degrees Fahrenheit. There was no relation between the dosage and either the temperature rise or the general course of the temperature curve. For example, the highest and lowest rise from the initial temperature, 3.5 and 0.6 degrees Fahrenheit, were obtained by nearly the same dosage, 0.013 g. and 0.016 g. per kilo body weight respectively. For animals receiving the solvent alone the maximum rises were, 2.5, 2.2, 1.7, 1.5, 1.2, 1.2, 1.0, and 0.4. The greatest temperature variations in the controls were from − 0.8 to + 1.0 of the initial temperature in Fahrenheit degrees. A composite curve of the variations in temperature of the animals of each series is given. The initial temperature before inoculation is taken as point of origin.



CURVE I. 1 cm. abscissa = 1 hour. 1 cm. ordinate = 1 degree Fahrenheit. Upper full line represents the composite curve of the temperature variations in the hematin animals. Middle dotted line is the composite curve of the solvent animals. Lower full line is the composite temperature curve of the control animals.

After intravenous injection of hematin solution in rabbits there is no resemblance in either temperature curve or in other respects to the malarial paroxysm in man. Both the solvent and the hematin solution cause an elevation of temperature in rabbits. There is no significant difference between the temperature curve of the hematin animals and that of the solvent animals.

Large volumes of the hematin solution, 30 c.c. to 40 c.c. that is over 0.04 g. per kilo body weight, kill rabbits almost immediately. Death is caused by intravascular precipitation of hematin with consequent embolism of the smaller vessels. The heart blood of animals autopsied immediately after death contains masses of granular hematin. The lungs are brown and their capillaries plugged with masses of granular hematin. If the animals

survive the injection several hours or longer there is a profound drop in temperature (as much as 6 degrees Fahrenheit) and numerous petechiæ are found in the heart muscle and in the gastro-intestinal tract. The intravascular precipitation of hematin can be reproduced in vitro by allowing the venous blood of a hirudinized rabbit and 0.5 per cent. hematin solution to flow together. The precipitation is not quantitative and its mechanism is not yet clearly established, but it is probably due to some constituent of the blood serum or plasma. Hematin itself is insoluble in blood serum of the rabbit. Injected intraperitoneally hematin is precipitated from alkaline solution in the peritoneal cavity of the animal.

In addition to the experimental work on rabbits, the blood sera of 19 patients with malarial organisms in the blood were examined for hematin. The blood was taken before and after the paroxysm and before the administration of quinine. In no case was hematin demonstrable. The quantity of hematin which can be detected spectroscopically in human blood serum in 7 cm. layers is less than 1 part in 3,000.

48 (865)

The effect of pituitary extract on the secretion of milk in the cow.

By **R. L. HILL** and **SUTHERLAND SIMPSON.**

[*From the Department of Physiology and Biochemistry, Medical College, and the Department of Animal Husbandry, College of Agriculture, Cornell University.*]

Ott and Scott (1), Schäfer and Mackenzie (2), Mackenzie (3), and Hammond (4) are agreed that the intravenous, intramuscular or subcutaneous administration of pituitary extract (posterior lobe) to lactating animals causes a marked increase in the rate of secretion of the mammary gland. The effect appears within twenty or thirty seconds after injection and lasts for three or four minutes.

In order to find out what effect the extract might have on the total quantity of milk per diem and on its quality Gavin (5) experimented with dairy cows under ordinary farm conditions; he

found that there was no change either in the amount or in the composition of the milk for the twenty-four-hour period.

We[1] have found that in the goat, within fifteen minutes after the injection, there is invariably an increase in the quantity of milk obtained and likewise in the percentage of fat. This has also been observed by Hammond. In one of our experiments the fat reached the high figure of 18 per cent. in the milk yielded after injection, while the normal for this animal was about 5 per cent. There is, however, a diminution in the quantity of milk obtained at the next milking period but the fat content remains above the normal for a day or two. The solids-not-fat of the milk do not appear to be affected.

The experiments which we desire to report on this occasion were made on the cow. Two animals at different periods of lactation were used. They were milked by hand, twice a day, at 6 a.m. and 5 p.m., and records were kept of the amount yielded and the percentage of fat for some days before and after the injection, as shown in the following tables:

TABLE I.

GUERNSEY COW; AGE 12 YEARS; WEIGHT 930 LBS.; LAST CALF BORN MORE THAN ONE YEAR AGO.

	Weight of Milk in Pounds.		Percentage of Fat.	
	Morning.	Evening.	Morning.	Evening.
January 28	5.9	4.5	4.8	5.2
29	6.2	4.8	4.8	5.2
30	5.4	5.4	4.6	5.1
31	5.6	5.0	4.6	5.3
February 1	5.8	5.0	4.0	5.3
2	5.9	5.4	4.3	5.2
3	5.8	.....	5.1	.....
4	3.3	5.4	4.8	.....
5	5.9	5.4	4.9	5.0

On February 3, at 4.30 p.m., this cow was milked dry; 4.7 lbs. of milk was obtained which contained 4.9 per cent. of fat. She was in heat and was being examined by a veterinary surgeon which caused some delay. At 5.15 p.m. a Ringer's solution extract of eight whole ox pituitary glands (anterior and posterior lobes) was injected into the external jugular vein, and at 5.30 the milk

[1] Our paper is in the press and will appear in the first number of the *Quarterly Journal of Experimental Physiology* for the current year.

was withdrawn again. The yield was 1.2 lbs. and the fat content 11.2 per cent. Between the injection and second milking the uterus had been washed out by the surgeon, so that the conditions under which this experiment was made were very unfavorable. At 5.45 p.m. the cow was milked again but only two ounces was obtained with a fat content of 9.5 per cent. On the next morning there was a marked falling off in the quantity of milk but the percentage of fat was about the normal, viz., 4.8 per cent.

On the evening of February 4 a control experiment was made as follows: The cow was milked dry at 4.45 p.m., and again at 5.45 p.m. The first yield was 5.4 lbs.—fat 5.9 per cent., and the second about one ounce,—fat 9 per cent., the result of "stripping."

TABLE II.

JERSEY COW; AGE 6 YEARS; WEIGHT 950 LBS.; LAST CALF BORN SEPT. 23, 1913.

	Weight of Milk in Pounds.		Percentage of Fat.	
	Morning.	Evening.	Morning.	Evening.
February 12	10.3	8.9	—	—
13	10.0	9.3	—	—
14	10.1	8.7	6.6	7.0
15	10.4	9.3	6.4	.....
16	6.0	10.1	5.4	.....
17	7.0	10.4	4.4	6.2
18	11.2	.....	6.6	.....

On February 15, at 5.15 p.m., this cow was milked dry and yielded 8.3 lbs. containing 7 per cent. of fat. The Ringer's solution extract of eight pituitaries (posterior lobes alone) was then injected into the external jugular vein and three minutes after the operation had been completed—at 5.30 p.m.—the udder was again emptied. At this second milking one pound was obtained with a fat content of 19 per cent. On milking a third time, 15 minutes later, not more than two ounces could be withdrawn; in this the percentage of fat was 10.5. Next morning (February 16) there was a marked drop in the quantity of milk and some diminution in the percentage of fat.

On the evening of February 16 a second experiment was made on this cow. At 4.45 p.m. the udder was completely emptied and yielded 8.9 lbs. of milk with a fat content of 5.5 per cent. Fifteen minutes later the cow was milked again when about one

ounce was obtained yielding 11.5 per cent. of fat—the result of "stripping." The injection was then made into the external jugular vein as on the previous evening, the same quantity of extract being used, viz., the equivalent of eight posterior lobes. About three minutes after the operation was finished, and fifteen minutes after the second milking, the udder was emptied a third time and now 1.2 lbs. of milk was obtained with a fat content of 14 per cent.

On the morning of February 17 the quantity of milk was again distinctly below the normal as also the percentage of fat.

The examination of the milk constituents other than fat has not yet been completed.

The conclusions to be drawn from the above experiments are that,

1. In the cow, the intravenous injection of pituitary extract (whole gland or posterior lobe alone) leads to an immediate secretion of milk very rich in fat. The effect, however, quickly passes off.

2. There is a corresponding diminution in the yield of milk at the next milking period, and to some extent in the percentage of fat, so that for the twenty-four hours there is practically no increase either in the total quantity of milk or of fat obtained.

We are indebted to the firm of Parke, Davis & Co. for the supply of the material which we have used.

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49 (866)

The cellular interpretation of anaphylaxis and immunity.

By RICHARD WEIL.

[*From the Cornell University Medical College, N. Y. City.*]

It has been shown in previous communications that the serological evidence is not in harmony with the current view that anaphylaxis is due to a reaction between the antibody present in the blood and the introduced antigen. This contention has now been confirmed with the help of the graphic method introduced by Dale, by means of the following experiment. A guinea-pig which is passively sensitized by the injection of 0.3 cubic centimeters of the serum of a rabbit immunized against horse serum, may be killed on the following day by an intravenous injection of horse serum. The uterine preparation also, on the second day, responds typically to the administration of horse serum. If, however, the antigen is applied immediately after sensitization, no reaction occurs, either in vivo or in vitro. The same failure to react follows the injection of relatively enormous amounts of the immune rabbit's serum, as for example three cubic centimeters. Hence, it is apparent that the presence of immune bodies in the circulating blood does not suffice to make a guinea-pig hypersensitive, but that these antibodies must first be bound by the body cells.

In the present paper I shall, furthermore, make a preliminary report upon a new method of studying the mechanism of anaphylaxis and immunity. Hitherto it has been customary to study these phenomena by means of the reaction to antigen, induced through the presence of antibody in the organism. The object sought by the method herein described is to permit of the identification of the antigen, as well as of the antibody, in the sensitized or immunized animal.

The essential feature of this method consists in the use of an immune serum as antigen. To illustrate: if a guinea-pig be sensitized by means of large doses of the serum of a rabbit immunized against horse serum, it has been shown that the passive sensitization to horse serum persists over a period of two weeks. During

the latter part of this period, however, an active sensitization develops towards rabbit serum. It becomes possible, therefore, to test the same uterus for the presence both of the antigen, and of the antibody thereto. The antigen is revealed by the reaction produced by horse serum, which demonstrates that the rabbit component still persists. The antibody is revealed by the reaction to normal rabbit serum. This condition is illustrated in Fig. 1.

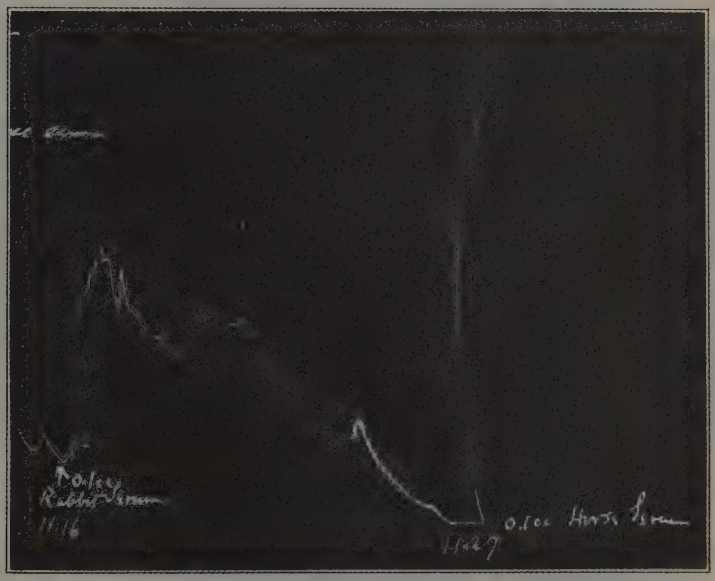

FIG. 1. Guinea-pig passively sensitized Jan. 4 by the intraperitoneal injection of 3 c.c. of the serum of a rabbit highly immunized against horse serum. Killed January 15, and uterine tracing taken. Reactions to both rabbit and horse serum.

In the same way, guinea-pigs actively sensitized or immunized against rabbit serum may be partially desensitized by means of an injection of the serum of a rabbit immunized against horse serum. The presence of the antigen in the sensitized cells is revealed by the reaction of the uterus to horse serum, while the reaction to rabbit serum shows that the autogenous antibody also persists. This fact is illustrated in Fig. 2.

The bearing of these data upon the interpretation of certain

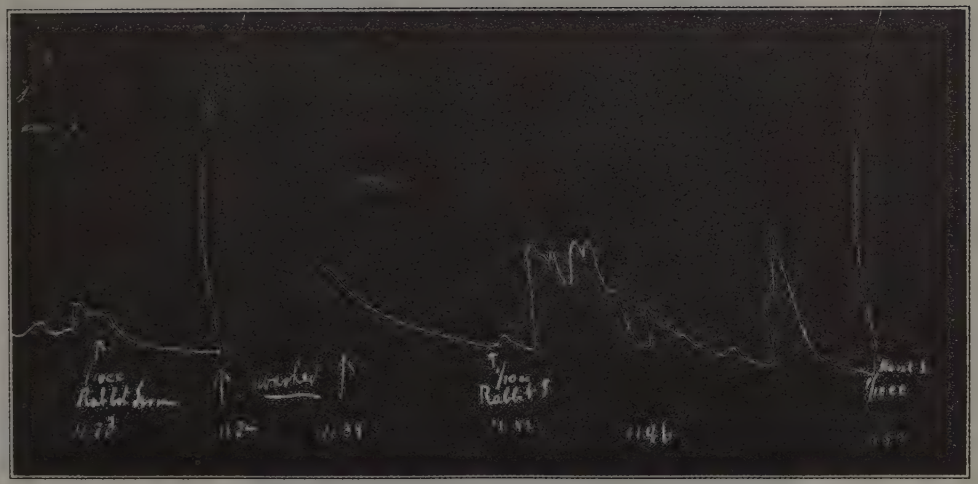

FIG. 2. Guinea-pig actively sensitized against rabbit serum November 11. Passively sensitized February 1 by the subcutaneous injection of 1 c.c. of the serum of a rabbit highly immunized against horse serum. Killed February 2, and uterine tracing taken. Reactions to both rabbit and horse serum.

phenomena of anaphylaxis and of immunity depends upon a third fact. It can be shown that a uterine preparation which has been partially desensitized by the addition of antigen is in a refractory, or "anti-anaphylactic" condition. Such a uterus no longer responds to the minute quantities of antigen which were at first effective, but can only be stimulated by relatively enormous amounts of antigen. This shows that the previous addition of antigen markedly lowers the reactivity of the antibody in the cells of the body. This is illustrated in Fig. 3.

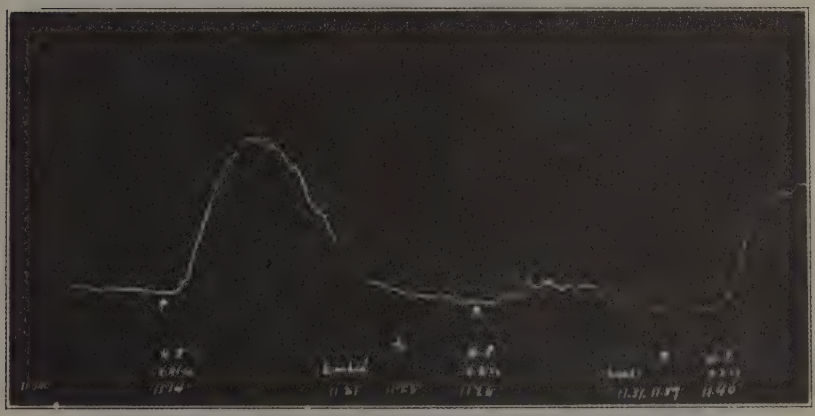

FIG. 3. Guinea-pig actively sensitized against horse serum November 21. Killed December 12, and uterine tracing taken. After preliminary additions of antigen, the organ responds only to large amounts of the latter.

The conclusion is therefore drawn that the resistance of immunized and of desensitized animals is in very great measure due to the presence of antigen in the cells, which markedly lowers the reactivity of the antibody in these cells. In course of time, by a spontaneous process of reaction, the antigen in the cells is destroyed, and the antibody again exists alone, or in preponderating amount, and the anaphylactic state recurs. In a previous paper I have shown that very large amounts of antibodies in the serum protect a guinea-pig only poorly against anaphylactic shock. The protective value of the antibodies in the blood of an animal

is, therefore, of relatively slight importance as compared with the presence of antigen in the cells. Therefore, it may be said that just as the presence of antibody in the serum is ineffective in making a guinea-pig hypersensitive, so, too, it fails adequately to explain the mechanism of immunity and of anti-anaphylaxis. The latter phenomenon, likewise, is predominantly cellular, and is due to the presence of antigen in the cell.

CONCLUSIONS.

1. The presence of antibody in the circulating blood alone does not make a guinea-pig hypersensitive. Such a result is achieved only by the presence of antibodies in the cells of the body.

2. The presence of antibody in the blood of the desensitized or of the immunized guinea-pig is only a subsidiary part of the mechanism of protection against anaphylactic shock. The presence of antigen within the cell is the effective factor, which so lowers the reactivity of the cellular antibody that additional antigen fails to produce the characteristic anaphylactic response.

3. By means of a special technique, the coëxistence of antigen and antibody within the same cellular tissue is demonstrated.

50 (867)

On the clinical value of the serum skin test in tuberculosis.

By **J. BRONFENBRENNER.**

[*From the Pathological and Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

In the work on Intraperitoneal Lysis of Tubercle Bacilli reported to this society last year by Dr. Manwaring and myself,[1] the conclusion was reached that tubercle bacilli injected in the peritoneal cavity of tuberculous guinea pigs undergo rapid destruction, due to the specific activity of peritoneal tissue cells which apparently did not depend on circulating antibody. In the experiments taken up this year, with the purpose of studying more closely the changes in the blood of tuberculous guinea-pigs, several interesting phenomena were found, to be reported in detail else-

[1] *Centrbl. f. Bact. Ref.*, Bd. 59, No. 12, p. 371, and *Journ. of Exp. Med.*, 1913, Vol. XVIII, no. 6, p. 601.

where, the main point of interest being that the subcutaneous injection of a mixture of the fresh blood of tuberculous guinea-pigs with exudate resulting from the intraperitoneal lysis of tubercle bacilli into a normal guinea-pig caused the appearance of a definite local reaction. Upon the analysis of the phenomenon, it was found that the peritoneal exudate could be replaced by a crude tuberculin, and the serum of tuberculous guinea-pigs could also be replaced by human tuberculous serum, and in this form it proved to furnish a very good method for early diagnosis of tuberculosis, the technique of which is as follows: Subcutaneous injection into a normal guinea-pig of 0.05 c.c. of a mixture of fresh tuberculous blood serum of human or animal origin (1 c.c.) with tuberculin (crude diluted—1 to 10—0.1 c.c.) left at room temperature for 2–3 hours, causes in 24 hours a local reaction similar in its aspect to a tuberculin reaction. The controls injected in similar way with the mixture of normal serum and tuberculin show no reaction. The property on which this serum skin test depends, appears in the blood of tuberculous guinea-pigs sometimes as early as the end of the first week after injection. When used as a diagnostic method with human sera, this test can be applied in all the stages of tuberculosis as long as the circulating antibodies exist free in the blood. I proved experimentally that tuberculous guinea-pigs within the last week or two of life fail to give this reaction, which finding seems to be true also for very advanced human cases. As compared with other skin tests used for diagnosis in tuberculosis, namely, one suggested by Schurmann,[1] who injects suspected material under the skin of guinea-pigs and subsequently tests them by regular tuberculin test, or the test suggested by Baureisen,[2] who injects suspected material under the skin of guinea-pigs made tuberculous two or three weeks previously, it seems that the method described by me covers better the different kinds of conditions in which the diagnosis may be called for. For the skin test described by Schurmann, it is necessary to have material containing virulent tubercle bacilli, which is not on hand in all cases of tuberculosis coming for diagnosis. The technique adopted by Baureisen has the dis-

[1] Schurmann, cited from *Z. f. Bakt.*, Vol. 59, p. 653.

[2] Baureisen, *Centr. f. Gyn.*, 1913, No. 23, p. 848.

advantage that it is necessary to keep on hand a number of tuberculous guinea-pigs; in addition the test was found non-reliable, as in case of sputum, for instance, the injection of a tuberculous or even normal sputum under the skin of a normal guinea-pig caused the appearance of a local reaction very similar to one appearing in a tuberculous guinea-pig. By the method described by me,[1] not only the serum of a patient, but also all kinds of pathological material can be examined by previously injecting it in a normal guinea-pig and subsequently examining the blood of this guinea-pig by the serum skin method described.

51 (868)

A preliminary communication on complement fixation test in tuberculoses with Besredka's antigen.

By J. BRONFENBRENNER.

[*From the Pathological and Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

At the suggestion of Professor M. Besredka and through his kindness in sending the tuberculin prepared by him as described in his communication before the Academy of Sciences,[2] I started a series of blood tests in tuberculosis. As the antigen contained egg yolk it was decided to carry in each case a control with a pure lipoid antigen (Noguchi).[3] In the first hundred cases I found a surprising number giving positive tuberculosis as well as positive Wassermann reaction. A special study of the possible coëxistence of the two diseases was made, and a solution of the problem was attempted by the following several ways. (1) Seven patients giving both W. R. and T. R. positive were subjected to a rigorous anti-syphilitic treatment, and at present five of them have lost the W. R., tuberculosis reaction persisting. (2) The presence of the two antibodies was proven by independent titration of each with five units of corresponding antigens. (3) It was found that the inactivation of serum containing both antibodies did not affect

[1] At the meeting of American Society of Bacteriologists, Montreal, January, 1914.

[2] *Comptes Rendus de l'Acad. des Sciences*, t. 156, p. 1633.

[3] Noguchi and Bronfenbrenner, *J. of Exper. Med.*, Vol. XIII, No. 1, 1911, p. 43.

the two similarly. (4) Finally one antibody was exhausted from the serum by repeated incubation with antigen and complement and subsequently the other antibody was proven to be still present.

The experiments described above carried various controls which are very complicated and will be fully described elsewhere.

In the course of experiments through the kindness of Dr. W. H. Park and Dr. A. A. McNeil, of New York, and Dr. Schildecker, Dr. Boyce and other members of the staff of this hospital, three hundred and twenty cases in all were examined up to date, comprising different conditions: typhoid, specific meningitis, tuberculosis, pernicious anemia, cancer, pneumonia, scarlet fever, lupus, diphtheria, syphilis, gonorrhea, trichinosis and various surgical conditions, with following results.

	W. + TB. +	W. + TB. −	W. − TB. +	W − TB. −	Total.
Serum	31	22	31	213	297
Spinal Fluid	4	6	0	13	23

The complete records of my cases will be published elsewhere. Here only the conclusions will be given:

1. That the serum reaction with Besredka's antigen seems specific.

2. That this reaction appears early in tuberculosis, yet disappears in the later stages of the disease.

3. That, as far as the material on hand was concerned, it seems that either syphilis as such or anti-syphilitic treatment markedly lowers the resistance of the patients against tuberculous infection.

4. As to other diseases their coëxistence with tuberculosis as indicated by this reaction does not seem to be frequent in any one condition.

5. In order to avoid any possible non-specific lipotropic reaction I propose to delipolyze the antigen which contains egg yolk. As regards the cases studied which gave both reactions, I invariably found the two reactions to exist independently.

In concluding this paper, I take special pleasure in expressing my indebtedness to Professor M. Besredka, whose kindness in placing the antigen in my hands, made the studies possible.

52 (869)

A consideration of certain foods and of proximity to a previous case as factors in the etiology of pellagra.

By J. F. SILER, M.D., P. E. GARRISON, M.D.,
W. J. MACNEAL, M.D.

[*From the Thompson-McFadden Pellagra Commission of the New York Post-Graduate Medical School.*]

A statistical study of the foods used and of the occurrence of pellagra in six mill villages, including about 5,000 persons, failed to reveal any consistent relationship between the use of any particular food and the occurrence of pellagra. A somewhat similar statistical study of the location of domicile of old cases of pellagra in relation to domicile of the remaining population in these same mill villages has shown that new cases of pellagra developed almost exclusively in persons living in the same house with such antecedent cases or in houses next door to them. In other words, the disease spread from a preceding or antecedent case as a center, a phenomenon which can be satisfactorily explained, in our opinion, only by assuming that pellagra is an infectious disease. Apparently it is not readily transmitted to any considerable distance.

53 (870)

The relation of methods of disposal of sewage to the spread of pellagra.

By J. F. SILER, M.D., P. E. GARRISON, M.D.,
W. J. MACNEAL, M.D.

[*From the Thompson-McFadden Pellagra Commission of the New York Post-Graduate Medical School and Hospital.*]

As we have observed in it our field studies, pellagra has spread most readily in communities in which unscreened surface privies were in use. In those portions of the city of Spartanburg, South

Carolina, equipped with a water-carriage system of sewage disposal, new cases of pellagra were relatively few. In two mill villages completely equipped with a water carriage sewer system, it was impossible to find cases of pellagra which had certainly originated there. Individuals suffering from pellagra contracted elsewhere were not lacking in these communities.

54 (871)

The fatal action of magnesium salts by absorption from the intestines and the resuscitation by calcium.

By J. AUER and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

A. Under ether anesthesia, the duodenum of a cat was exposed, ligated, a cannula inserted and fixed in the direction of the small intestines and the abdomen closed so as to permit the rubber tubing part of the cannula which was closed by a hemostatic forceps to protrude. The animal was then permitted to come out from the influence of ether and was wide awake. Ten c.c. of a 20 per cent. solution of $MgCl_2$ per kilo of body weight were now injected through the rubber tubing into the small intestines. The animal died after twenty five minutes by respiratory paralysis without any asphyctic convulsions; the heart continued to beat a little longer.

B. Another cat in which preparation and procedure was the same as in *A*. But when the respiration began to fail, artificial respiration by the pharyngeal method of Meltzer was started and through a venous cannula ten c.c. of a 2.5 per cent. solution of $CaCl_2$ was slowly injected. Spontaneous respiration soon appeared and the animal was resuscitated.

The experiments show, against the generally accepted view, that magnesium salts are rapidly absorbed from the intestines. They show further that the method of injecting magnesium salts into the small intestines after laparotomies as recommended by McCosh and practiced by some surgeons contains an element of considerable danger.

55 (872)

The convulsant action of strychnin and morphin in cardiectomized frogs after destruction of the anterior lymph hearts.

By T. S. GITHENS and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

Meltzer has shown several years ago that in cardiectomized frogs strychnin causes convulsions nearly as well as in normal frogs and morphin causes convulsions even a good deal sooner and with smaller doses. We have shown later that low temperature is an important factor in the success of these experiments. About a year and a half ago, Abel, while admitting the facts, made the statement that the success in these experiments depends upon the activity of the anterior lymph hearts. At the meeting of December, 1912, Meltzer demonstrated cardiectomized frogs with destroyed lymph hearts in strychnin convulsions. The method of complete evisceration employed in these experiments produced, however, such a complete shock that there was a success only in a certain percentage of the experiments. We now employed another method. After destruction of the anterior lymph hearts in normal anesthetized frogs, the animals are permitted to fully recover from the anesthetic and the operation, and the blood heart is removed either a few hours later on the same day or next day. The success is now nearly 100 per cent. The animals have, of course, to be kept at a fairly low temperature. The success is now positive although practically all the injections are made now in the lymph sacs surrounding the thigh and the solution has to travel a longer distance before it reaches the upper end of the cord. You see here frogs in strong tetanus which received strychnin or morphin several hours ago. After evisceration you can see now that the anterior lymph hearts were destroyed. It is evident that the anterior lymph hearts have nothing to do with the convulsive action of strychnin and morphin in cardiectomized frogs.

56 (873)

The depressive action of magnesium sulphate and sodium oxalate and the rapid antagonistic action of calcium.

By F. L. GATES and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

A. At the October meeting we reported that the effect of a combination of ineffective doses of magnesium sulphate and sodium oxalate is equal to that of an effective dose of magnesium sulphate alone, except that the anesthetic effect due to the combination of the two salts is of greater duration. Each of these two rabbits received about two hours ago 0.8 gm. magnesium sulphate and 0.2 gm. sodium oxalate per kilo body weight, subcutaneously. You see, they are still deeply anesthetized and paralyzed. Of the other two rabbits one received 0.8 gm. magnesium sulphate and the other 0.2 gm. sodium oxalate per kilo body weight subcutaneously. You see they behave practically normally.

B. Now each of the anesthetized animals receives through the ear vein, about 8 c.c. of a 2.5 per cent. solution of calcium chloride. You see that the respiration becomes deeper and more rapid almost immediately and within a minute of the beginning of the injection they turn over and sit up.

57 (874)

The pulmonary reaction to B. pyocyaneus.

By MARTHA WOLLSTEIN and S. J. MELTZER.

[*From the Laboratory of the Rockefeller Institute.*]

Intra-bronchial insufflation of broth cultures of *B. pyocyaneus* in doses of 10–15 c.c. were more fatal than any other organism thus far used, except *B. prodigiosus*.

The lesion produced was that of a lobular pneumonia with intra-alveolar hemorrhage and fibrinous pleurisy. The bacilli were recovered from the heart's blood and from the lungs.

Immunization experiments were successfully carried out, increasing doses of 1 to 15 c.c. being administered. Dogs survived such doses, and developed agglutinins to *B. pyocyaneus*.

In animals which survived ten days or longer, areas of unresolved pneumonia and thickened pleura were the rule.

58 (875)

A method for the separation of lipins from lipin extracts.

By JACOB ROSENBLOOM, M.D., PH.D.

[*From the Biochemical Laboratory of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

Owing to the solubility of lipins in ether and alcohol we have a basis for the isolation of these substances. To separate them from their ether and alcohol solution we have several methods. As a rule these methods are expensive as most of the solvent is lost, they require considerable time and in some of the methods heat is applied, a bad procedure on account of the labile nature of some of these substances.

I have devised a method which gives good results, allows the solvent to be regained and takes little time without using heat. It is based on the fact that lipins are *insoluble in water*. The method is as follows; to the ether or alcohol extract of the lipins add cold water containing 0.5 per cent. of sodium chloride,[1] till no further precipitation occurs. The water should be added slowly without shaking or stirring, otherwise some of the lipins will be emulsified. Any of the precipitate not coming to the surface may be obtained by filtration.[2] It will be found that this precipitate contains the lipins and if one wants to obtain the phospholipins from the precipitate, simply wash thoroughly with acetone until no residue is obtained, when the washings are evaporated to dryness. The acetone removes all the lipins except the phospholipins.

[1] The addition of sodium chloride to the water helps to flocculate the lipins and by raising the specific gravity of the solution, allows the lipins to come to the surface, where they may be skimmed off by means of a spoon. Percentages of sodium chloride under 0.5 per cent. do not give good results.

[2] The solvent can now be obtained by distilling it from the filtrate.

In the presence of much chromlipin (lipochrome) this method does not work so well as it requires from 12 to 24 hours for the insoluble lipins to separate out and as a rule the precipitate goes to the bottom of the container instead of rising to the surface. It appears from this observation that the presence of chromlipin in some way changes the physico-chemical conditions of the associated lipins.

59 (876)

Note on the effect of the internal secretions upon the volume of the pancreas.

By ISAAC OTT, M.D., and JOHN C. SCOTT, M.D.

[*From the Laboratory of the Medico-Chirurgical College of Philadelphia.*]

We have studied the action of infusions of the various dried glands upon the volume of the pancreas. The animals used were etherized cats. The injections were made per jugular. The volume of the pancreas was registered with a modified piston recorder.

Infundibulin (pituitrin) causes a marked increase in the volume of the pancreas. Adrenalin produces a decrease in volume for a short time and then an increase. The pineal gland infusion increases the volume. Infusion of thyroid momentarily decreases and then increases the volume. Iodothyrin also increases the volume. Thymus does the same.

Secretin depresses blood-pressure for a short time and increases the volume of the pancreas to a marked extent.

Infusion of pancreas decreases the volume for a moment and then increases the volume of the pancreas. The renal cortex decreases the blood-pressure for a moment, but increases the volume of the pancreas to a marked extent.

Prostatic infusion had no effect on pancreatic volume. Tonsillar infusion lowered blood-pressure and slightly increased volume of pancreas.

Mammary gland infusion decreased blood-pressure for a short time but caused a marked increase in pancreatic volume.

Spleen infusion had no effect on volume of the gland. The same may be said for orchitic extract.

Parathyroid infusion decreases blood-pressure for a short time, also decreases pancreatic volume for a short period, and then greatly increases it.

Infusion of corpus luteum decreases blood-pressure momentarily and increases the volume of the pancreas.

Pineal infusion increases the flow of pancreatic juice.

60 (877)

The origin of the cardiac impulse in the turtle's heart.

(PRELIMINARY COMMUNICATION.)

By W. J. MEEK and J. A. E. EYSTER.

[*From the Physiological Laboratory of the University of Wisconsin.*]

Recent work has emphasized the sinoauricular node as the seat of origin of the cardiac impulse in the normal heart. This structure must be considered anatomically as forming a connection or junction between remains of the primitive sinus, which has disappeared as a separate chamber in the mammalia, and the auricle. Since indications of the presence of a sinoauricular junction, composed of tissue differentiated histologically from the ordinary cardiac muscle, is found not only in those hearts in which the sinus has disappeared as a separate chamber, but also in the amphibia and reptiles, where a separate sinus venosus is present, it becomes of essential importance to determine whether or not in these animals the heart beat arises as it does in the mammalian heart, that is in the sinoauricular junction. This problem may be attacked by determining that region which shows initial electric negativity when connected with the string galvanometer. We have had this problem in mind for some time, but have been unable to proceed with it because of the difficulty in securing material with sufficiently large hearts to test this point satisfactorily. Recently we have been able to secure one large turtle in which the heart was of sufficient size. In this the right half of the sinus was 35 millimeters long and 20 millimeters wide at its

junction with the left half. We compared the onset of negativity at the sinoauricular junction with the right and left halves of the sinus, and found the junction to precede in negativity. It must be borne in mind, however, that in the exposed heart, automaticity and conductivity may sometimes be abnormal, and a fairly long series of observations will be necessary in order to determine without question, the normal origin of the impulse. We have arranged for a supply of large turtles in order to continue the work, but shipment of these to northern points can be made only in late spring or summer. Since our work is thus temporarily delayed and since we feel the observation, if it can be confirmed by other experiments, is of fundamental importance, we have been led to make a report of this single experiment at the present time.

61 (878)

Further observations on the physiological properties of the lipins of the egg yolk.

By E. V. McCOLLUM and MARGUERITE DAVIS (*by invitation*).

[*From the Laboratory of Agricultural Chemistry of the University of Wisconsin.*]

We have continued our former studies[1] which have shown that rats which have grown as far as possible on a diet of casein, dextrin and inorganic salts, can make a new growth when small amounts of the ether extract of butter or of egg is added, while they are unable to do so when lard, olive oil, lecithin or cholesterin is added instead of the ether extracts mentioned. These have since been fully confirmed by Osborne and Mendel who have obtained similar results when unsalted butter[2] or purified butter fat[3] was employed.

We have now observed that ether, or petroleum ether extract of *boiled eggs* possesses the same physiological property of inducing a new growth as does the extract previously described. The active principle, whose chemical nature is still unknown, is therefore resistant to heat, and is soluble in petroleum ether as well as

[1] *Journal of Biological Chemistry*, Vol. 15, p. 167 (1913).

[2] Osborne and Mendel, *Journal of Biological Chemistry*, Vol. 15, p. 311 (1913).

[3] Osborne and Mendel, *Journal of Biological Chemistry*, Vol. 16, p. 423 (1913).

ether. Experiments now in progress seem to indicate that the acetone extract of egg yolk possesses in some degree at least the same power to induce new growth in rats whose growth has become suspended after long feeding with diets of purified food substances.

62 (879)

The experimental production of hyaline casts by injections of magnesium salts.

By F. L. GATES (*by invitation*).

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In view of the urinary findings after magnesium-ether anesthesia in human cases, just reported to you by Dr. Peck, Dr. Meltzer suggested that we try to produce hyaline casts in animals by injections of magnesium salts.

We have used rabbits, monkeys, and dogs, but the results to be reported tonight relate only to experiments on dogs. Epsom salt in sublethal doses, with and without ether by insufflation, was given to dogs intramuscularly and intravenously. In all cases many hyaline casts appeared. They were most abundant from 3 to 6 hours after the injection and disappeared in 24 or at most 48 hours. No epithelial or granular casts were found and no measurable albuminuria occurred. Ether alone did not produce casts and ether and magnesium produced no more than magnesium alone. In one experiment, magnesium chloride gave the same results as the sulphate.

Our essential point is that hyaline casts in large numbers can be produced in dogs at will by injections of magnesium salts. Epithelial and granular casts and albuminuria are not present and the appearance of the hyaline casts is only temporary.

63 (880)

The occurrence of casts in the urine following magnesium sulphate ether anesthesia (Meltzer).

By CHARLES H. PECK (*by invitation*).

[*From The Roosevelt Hospital, New York City.*]

The report relates to the occurrence of casts in the urine following operation under magnesium sulphate-ether anesthesia, in a series of five cases observed by Drs. Meltzer and Peck at the Roosevelt Hospital.

Three cases showed hyaline casts in the urine which disappeared after 48 hours and did not reappear. One of the three showed a faint trace of albumin; the other two, no albumin.

Two cases showed hyaline and granular casts and a trace of albumin, one persisting for six days, one for two days or more, being normal on the fifth day.

None of the cases showed any later evidence of kidney irritation. In all of the cases magnesium could be demonstrated in the urine for several days. In none of the cases were casts present in the urine before operation.

64 (881)

The experimental production of an early stage of extrauterine pregnancy

By LEO LOEB.

[*Barnard Free Skin and Cancer Hospital, St. Louis, Mo.*]

In former experiments I had shown that it is apparently impossible to produce experimentally an extrauterine pregnancy in the guinea pig.[1] Neither ligation of the fallopian tubes at the juncture with the uterus, nor incision into the tubes or wall of the uterus at various periods of the sexual cycle led to the development of an extrauterine pregnancy. In another series of experi-

[1] Leo Loeb and John W. Hunter, *University of Pennsylvania Med. Bulletin*, Dec., 1908.

ments I had shown that in the guinea pig only the mucosa of the uterus is able to produce a decidua under the influence of experimentally applied stimuli. In the human organism where extrauterine pregnancy occurs, decidua can to some extent form also at certain places outside of the uterine mucosa. It is, therefore, probable that one of the factors upon which the development of an extrauterine pregnancy depends is the capability of the tissue, into which the ovum penetrates, to develop decidual tissue which presents to the ovum an adequate soil for development.

In an experiment carried out recently I was able to show that it is possible to produce experimentally the first stages of an extrauterine pregnancy in the guinea pig, that however the development of embryo and placenta outside the uterus ends prematurely.

Incisions were made into the uterus of a guinea pig two days, sixteen hours after copulation. The incision reached upwards through the greater part of both horns. Eighteen days after copulation, examination of ovary and uterus showed that a new ovulation had taken place approximately three days previously, fifteen days after the preceding copulation. On the peritoneal side of the left horn of the uterus, near the fallopian tube was embedded superficially in the tissue a young embryo in which structures appear which resemble the neural canal, coelomic and enteric cavities and the anlage of the blood vessels. These structures are somewhat distorted. They are surrounded by placental formations, namely, syncytia, plasmodia and small cuboidal cells. The giant cells penetrate into the surrounding tissue of the uterine peritoneal coat and ensheath especially the blood vessels, substituting their wall; the small cuboidal cells supply the lining of cavities, into the interior of which they send papillary processes. Frequent mitoses are present in the cuboidal cells; occasionally a mitosis can also be seen in a giant cell. The plasmodia surround externally these coats of cuboidal cells. Some hemorrhages surround the whole structure and a little connective tissue of the peritoneal coat of the uterus covers it. Decidual tissue is entirely absent. There is no reaction on the part of the surrounding host connective tissue or of the blood vessels. We have without doubt to deal with an ovum which escaped from the upper end of the

uterine cavity through the incision made in the uterus two days, sixteen hours after copulation. The ovum migrated around the peritoneal covering of the uterus and embedded itself superficially in the connective tissue of the peritoneum. Here it developed and is still developing at the times of examination, as the existing mitoses indicated. It produced certain placental structures which joined the surrounding connective tissue and blood vessels. It is not very probable that an ovum leaving the ovary at the time of the last ovulation three days ago could within so short a period of time produce these structures after the passage through the tubes. In this case we would have to deal with parthenogenetic development of the ovum. It is very much more probable that we have to deal with the extrauterine fixation and development of an ovum leaving the uterine cavity through the incision made a few days after the previous ovulation, the cut in the upper part of the uterine horn having been made exactly at the time when the ovum was approaching the uterus after its passage through the fallopian tube. In this case we would have to deal with a very slow and very much delayed development of the embryo, due to the unfavorable conditions under which the ovum develops.

These findings are of importance: first, because they confirm our previous findings, that connective tissue other than that of the uterine mucosa is unable to produce decidua in the guinea pig. While it is under these unfavorable conditions possible for an ovum to undergo the early stages of development, lack of the proper response on the part of the host tissue (lack of decidual reaction) renders the development of the later stages of extrauterine embryonal development impossible.

2. In contact with the connective tissue and vessels of the host the ovum develops mainly certain parts of the fetal placenta, namely syncytia and plasmodia and layers of small cuboidal cells. These structures correspond to certain parts of the normal placenta of the guinea pig which lie in the periphery of the embryo and enter under ordinary conditions into relations with the maternal part of the placenta. Certain parts of the trophoblast and the embryo proper develop only in a rudimentary manner under these conditions.

3. The embryonic and placental structures developing outside

of the uterine cavity resemble in every respect closely the structures which I found[1] in about ten per cent. of the ovaries of young guinea pigs and which I interpreted as ova developing parthenogenetically in the ovaries.

Previously some investigators as well as myself had described the first irregular cleavages in the ova in atretic follicles of certain young guinea pigs. My later observations made very probable the development of the ova to the stage in which the anlage of the nervous system and parts of the fetal placenta are formed. In this case also young guinea pigs are more favorable to the development of the ova.

Our new observation confirms the correctness of our interpretation of the structures found in the ovaries and we can, therefore, state with certainty that in a considerable number of the ovaries of young guinea pigs a relatively far-going parthenogenetic development of the ova takes place.

[1] *Arch. f. Mikrosk. Anat.*, Bd. 65, 1905; *Journal Am. Med. Assn.*, May 6, 1911; *Zeitschrift f. Krebsforchung*, Bd. 11, 1912.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Fifty-eighth meeting.

University and Bellevue Hospital Medical College, April 15, 1914.

Vice-President Gies in the chair.

65 (882)

The influence of the diaphragm descent on the movements of the heart.

By Carl J. Wiggers.

[*From the Physiological Laboratory of Cornell University Medical College, New York City.*]

If the upper three fourths of the sternum, together with the corresponding ribs are resected, so that the heart remains intact within the pericardium attached to the diaphragm and, in addition, the severed sterno-pericardial bands are fastened to a wire substituted for the sternum, the movements of the heart correspond to those within the closed chest. The base descends, the apex rotates anteriorly and in so doing moves downward slightly. In such experiments the effect of diaphragm descent may be produced by discontinuing artificial respiration with the lungs in a state of partial inflation, in which event the animal resumes natural breathing. The phrenic nerves may also be stimulated during periods of apnea vera.

By attaching strings to four or five separate points of the heart, large vessels and diaphragm and connecting them to receiving tambours which in turn transmitted their pulsations to recording tambours, it was possible to determine the effect of diaphragmatic traction graphically. The following observations were made.

1. During descent of the diaphragm, the posterior portions of the heart and the venæ cavæ descend more than the anterior portion of the base, while the anterior aspects of the apex move forward and as a result often slightly upward. The right and left borders of the ventricle move toward the right.

2. The movement of the heart to the right does not occur after severing the left phrenic nerves nor upon stimulating the right phrenic nerve. It is, therefore, due to a traction of the left sheath of the pericardium.

3. A descent of the base of the ventricles, the auricles and venæ cavæ occurs after the entire pericardium is severed from the diaphragm. Direct experiments show that this is due in part to a traction upon the inferior vena cava making it larger and narrower.

4. The descent of the base of the heart persists after the vena cava is clamped and divided. This results from a traction upon the ligamentum pulmonale (a double fold continuous with the pleura pulmonalis, and passing downward from the root of the lung to its vertebral and diaphragmatic attachments) which causes the roots of the lungs, the pulmonary vessels and through these the base of the heart to move downwards.

66 (883)

Experiments dealing with the relation of the sinus node to the effects of stimulation of the vagus nerves.

By ALFRED E. COHN.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

Experiments by a number of investigators on the sino-auricular node have warranted the conclusion that this structure is responsible for stimulus production and the maintenance of the rate of the heart. Flack thought that this node formed a station in the pathway, both of the vagus and of the accelerator nerves, and that through its agency they exercised their influence on the heart. He attempted to show that exclusion of the node from function consequently interrupted impulses passing over the nerves.

It is the purpose of this paper to report that a large number of experiments have been performed on dogs to determine the relation of the vagus nerves to the sinus node. The experiments were performed under ether; a small opening in the chest was made by resecting one or two ribs, and both vagus nerves were dissected, cut and laid on shield electrodes. Records were taken electrocardiographically. The results of stimulation of both vagus nerves were first registered. Then the sinus node was carefully clamped off with a suitable T-shaped clamp, all the tissue surrounding the node being crushed. Stimulation of both vagus nerves was repeated and records were obtained.

In the greater number of experiments, it could be shown that stimulation of the vagus nerves after clamping the node was effectual in producing effects on the rate and on the rhythm of the heart in a way similar to that seen before the clamp was placed. In a number of cases the effect of stimulation was more profound after than before the clamping. The conclusion is therefore warranted that the sinus node does not represent a simple relay in the course of the vagus nerves.

These experiments will be published in detail later, and the histological examinations of the areas at which the clamp was applied will be reported.

67 (834)

The energy requirement of the new born.

By **H. C. BAILEY** and **J. R. MURLIN.**

[*From the Physiological Laboratory of Cornell University and the Maternity Wards of Bellevue Hospital.*]

To find whether it would be desirable from a physiological standpoint to furnish some artificial food together with the colostrum during the first three days of life, the energy requirement of the newborn was ascertained by means of a respiration incubator.

Series of cases from the wards of Bellevue Hospital show that the initial weight loss averages over 250 gm. and that this loss is increased by lengthening the interval of feedings and lessened by

the supplementary feeding of a milk mixture similar to colostrum. The infants were able to take the extra feedings without any disturbances of digestion.

Mr. Frank Gephart, of the physiological laboratory, made analyses of five specimens of second- and third-day colostrum and found the utilizable heat value averaged 65 calories per 100 c.c. While the food value of the colostrum is great, our own observations and many reports from the literature show that the average amount of breast secretion is 14 c.c. on the first day, 77 c.c. on the second day, 173 c.c. on the third day and from that time on gradually increasing to 372 c.c. on the sixth day, hardly, in the first three days at least, an amount that would give the infant much nourishment.

With the respiration apparatus of Benedict connected to an airtight chamber placed within a Freas electric constant temperature incubator of special construction, the carbon dioxide eliminated and the oxygen absorbed were measured under proper temperature conditions.

Nineteen observations were made on six newborn infants varying in age from 6 hours to 12 days. From the results were obtained the respiratory quotients and by the indirect method, using the method of Zuntz and Schumberg, the heat production was calculated.

SUMMARY OF THE RESULTS.

The respiratory quotient reaches as high as 1.0 on the first day of life and indicates the combustion of carbohydrates. Thereafter it drops to 0.67 on the second day and remains in the neighborhood of 0.70 for the following two days indicating a condition of starvation and the combustion of fat. After the milk secretion is well established the quotient reaches 0.90, which is the normal for a mixed diet.

The average food requirement for combustion alone during the first four days of life for a large infant weighing 4.5 kg. is 1.7 calories per kilogram per hour; and for a small infant weighing 3 kg. is 2.0 calories per kilogram per hour.

Comparing the calories per 100 c.c. of samples of colostrum with this requirement it is evident that the infants would have to

suckle at least 30 c.c. at ten feedings. This is an amount that the breast does not secrete, at least until the fourth day. Feeding the newborn infants for the first three days, in addition to the breast secretion, a formula of about the same composition as colostrum would appear to be a logical proceeding not only to fulfill the energy requirement but also to supply the water lost.

Even after the breasts are secreting a considerable quantity of milk it would seem to be rational to feed these babies as often as every two or two and one half hours during the day or eight or nine feedings in all.

68 (885)

The influence of phenylquinolin carbonic acid (atophan) and of radium emanation upon the uric acid concentration of the blood.

By MORRIS S. FINE and ARTHUR F. CHACE.

[*From the Laboratory of Pathological Chemistry and the Department of Medicine, New York Post-Graduate Medical School and Hospital.*]

The prompt and marked increase in the uric acid output following the administration of 2-phenylquinolin-4-carbonic acid (atophan) has been repeatedly demonstrated. More recently, Folin and Lyman[1] reported a concomitant diminution in the uric acid concentration of the blood, which suggested an increased renal permeability as an important factor in the physiological action of atophan.

In the course of our studies with atophan, begun more than a year ago, we have endeavored to ascertain, (1) the promptness with which the decrease in blood uric acid is brought about; (2) the minimum to which it can be reduced; and (3) the rapidity with which the initial concentration is regained after the administration of atophan has ceased.

We have observed a marked decrease in the uric acid concentration of the blood after a two day period with atophan; and are attempting to determine more exactly the speed of this reaction.

[1] Folin and Lyman, *Jour. Pharm. and Expt. Ther.*, IV, p. 539, 1913.

The minimum uric acid concentration of the blood under the influence of atophan varied among twelve individuals (normal, gouty and arthritic) from 0.7 to 2.0 mgm. per hundred grams of blood (initial values — 2.0 to 5.7). These minima are apparently irreducible. In one case, the daily administration of 4 grams of atophan for a period of 45 days failed to lower the uric acid below 0.7 mgm.

The initial concentration is restored within at least 2 to 4 days after the cessation of the use of atophan.

Our studies upon the uric acid concentration of the blood under various conditions are being continued, and will include the influence of colchicum and the salicylates.

With a radium emanation concentration of 2 to 4 M. U. per liter of air, Gudzent[2] claims to have caused uric acid to disappear from the blood for long periods of time. With our radium equipment, designed to yield 4 to 6 M. U. per liter, there was no noteworthy change in the uric acid concentration of the blood. We desire, however, to reserve definite conclusions until we have completed further studies with radium emanation.

69 (886)

Some structural transformations of the blood cells of vertebrates.

By G. L. KITE (*by invitation*).

[*From the Henry Phipps Institute of the University of Pennsylvania.*]

The chief results of a study of structural transformations in the living blood cells of fishes, amphibians, reptiles, birds, and mammals are given in this paper.

The blood cells were usually prepared for observation by mixing fresh blood with Ringer's fluid containing sufficient hirudin to prevent coagulation.

Dark field illumination proved to be satisfactory for the observation of structural transformations in blood cells.

The following five forms or phases may be assumed by vertebrate white blood cells:

1. The hyaline surface phase which is characterized by a hyaline change and great increase in viscidity of the surface layer.

[2] Gudzent, *Berl. klin. Woch.*, XLVIII (2), p. 2098, 1911.

2. The hyaline phase in which the globular component is localized in a small area of the extremely hyaline cytoplasm.

3. The fine pseudopodial phase which is characterized by the protrusion of fine long motionless protoplasmic processes.

4. The ciliated phase of the white blood cell shows numerous beating cilium-like processes.

5. The flagellated phase is distinguished by the presence of numerous long protoplasmic processes which exhibit the rapid undulatory movement characteristic of flagella.

Notable redistribution phenomena have been observed in erythrocytes of both warm- and cold-blooded vertebrates as well as the formation of either cilium-like or flagellum-like processes. White blood cells or erythrocytes which have numerous rapidly moving protoplasmic processes may be actually free-swimming.

Similar structural transformations have been observed in myelocytes, "pyrrhol" cells, and in cells from inflammatory exudates.

The rôle of structural transformations of white blood cells in phagocytosis has been investigated with interesting results.

70 (887)

Colloidal nitrogen in diabetes.

By GEORGE B. WALLACE.

[*From the Laboratory of Pharmacology, University and Bellevue Hospital Medical College.*]

The so-called colloidal nitrogen fraction of the urine has normally a fairly constant relationship to the total nitrogen, forming from 1 to 4.5 per cent. of the latter. In a number of pathological conditions, the percentage may be greatly increased, rising in some instances to 35 per cent. In a study of this subject by Pribram and collaborators, it was found that in severe diabetes, among other diseases, the percentage of colloidal nitrogen was especially high. Pribram interprets this as an evidence of abnormal or lessened liver function and associates it with the occurrence of diabetic coma.

I have determined the colloidal nitrogen fraction in two cases

of diabetes. The first was in diabetic coma, which had a fatal outcome. The analysis of the urine collected on the second day of coma gave the following figures: Total nitrogen, 7.78 g.; colloidal nitrogen, 0.22 g. The colloidal nitrogen fraction was 2.87 per cent. of the total nitrogen, a normal relationship.

The second case was one of moderately severe diabetes, with a moderate degree of acetonuria and glycosuria. One analysis gave: Total nitrogen, 14.84 g.; colloidal nitrogen, 0.91 g. The colloidal nitrogen was 6.2 per cent. of the total nitrogen.

An analysis made ten days later on the urine of this patient showed: Total nitrogen, 17.9 g.; colloidal nitrogen, 0.43 g.; colloidal nitrogen = 2.4 per cent. of total nitrogen.

According to these figures, which agree with some reported by Wolf and Marriott, there appears to be no definite relationship between the severity of diabetes and the urinary colloidal nitrogen percentage. It is quite possible, however, that the colloidal substance may fail to be increased in the urine because of faulty kidney elimination and that the blood content may be considerably above the normal without the urine showing any evidence of this.

71 (888)

A depressor substance in fecal extracts.

By GEORGE B. WALLACE and MILLS STURTEVANT.

[*From the Laboratory of Pharmacology, University and Bellevue Hospital Medical College.*]

In the course of a study on the relationship of certain symptoms to intestinal disturbances we prepared a ten per cent. watery extract of dried feces and injected 3 c.c. of this intravenously into a dog. The injection was followed by a sudden, sharp fall in blood pressure, with a return to normal level within five minutes. Subsequent injections into the same animal produced a similar fall only when the dose was markedly increased. Extracts of both dried and fresh feces from various individuals have been prepared and produce similar results on injection. The fall in pressure may reach seventy per cent. With the fall there is seen some quickening in heart rate and at times an irregularity in rhythm.

Oncometer registration of the kidney and leg volume shows a decided lessening in volume. The intestinal volume on the other hand shows usually a slight rise. A myocardiogram shows no weakening of carciac contractions sufficient to explain the fall in pressure, which therefore seems due to a dilatation of the mesenteric vessels. A further analysis of the point of attack in this depressor action has not been made as yet.

Concerning the nature of the depressor substance, the following facts have been obtained. It is not destroyed by boiling; nor removed by heat and acetic acid; after burning the dried feces, an extract of the ash has no action; the freezing point determination of the ten per cent. extract gives a Δ of approximately 0.3°. The substance does not dialyze. It appears therefore to be of colloidal nature.

72 (889)

The energy metabolism of normal and marasmic children with special reference to the specific gravity of the child's body.

By J. R. MURLIN and B. R. HOOBLER.

[*From the Physiological Laboratory of Cornell University Medical College and the Children's Wards of Bellevue Hospital, New York City.*]

A study of six normal and two atrophic children was made with the respiration incubator. The child was weighed and the approximate specific gravity was determined by Pfaundler's method immediately afterwards. The child was then placed on a metabolism bed, was given a good feeding[1] and the bed was placed in the respiration chamber. The respiratory exchange was accurately determined in two consecutive periods of one hour each on each of several observation days. The urine was collected in 24 hour periods and the heat production was calculated from the oxygen absorption and the nitrogen in the urine after the method of Zuntz and Schumberg. The temperature of the incubator was kept at about 27° C. average, this having been found to be most

[1] The amount of this feeding for different cases was nearly the same reckoned in calories per kilogram of body weight.

conducive to sleep under the circumstances. As a rule, the child slept perfectly the first hour and several times slept throughout both respiration periods.

A graphic record of the pulse was obtained from a blood pressure cuff on the thigh and a record of the respirations from a small pneumograph placed about the chest. The pressure in the cuff was kept well below the diastolic pressure and did not interfere either with the circulation in the leg or with the repose of the child. These recording devices served also to record grosser movements when the child moved any part of the body or cried.

Comparing only the best sleeping periods it was found that the metabolism in different children was much more nearly proportional to the weight than to the surface area and when the weight was first multiplied by the specific gravity the agreement was even better.

TABLE SHOWING ENERGY PRODUCTION PER HOUR.

Child No.	Age, Mos.	Weight, Gms.	Cal. per Kgm.	Sp. Gr. *d.*	Cal. per hr. Wt. × *d.*	Cal. per Sq. M.
I. Normal boy	2	5,690	2.44	0.973	2.51	35.51
			2.52		2.59	36.58
II. Normal boy	2	4,634	2.44	1.034	2.31	32.53
		4,350	2.47	1.033	2.39	32.03
III. Under weight boy	3	4,115	2.61	1.006	2.60	34.57
		4,147	2.54	1.005	2.53	33.20
IV. Atrophic boy	3	2,462	2.97	1.108	2.68	32.41
			2.92		2.64	
		2,515	2.94	1.118	2.63	32.51
			2.94		2.64	
V. Normal girl	10½	9,465	2.64	1.026	2.57	45.37
VI. Normal boy	12	9,555	2.80	1.029	2.73	48.40

One atrophic child was in the last stages of marasmus when the observations were made and gave a much lower metabolism than the others. One normal child was very nervous and failed to sleep perfectly. These two are not included in the table.

73 (890)

Parathyroid hypertrophy and hyperplasia in fowls.

By DAVID MARINE (*by invitation*).

[*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.*]

Physiological overgrowth of the parathyroid glands in mammals has been very rarely observed. Erdheim,[1] Bauer[2] and Strada[3] have recently described its occurrence in man in association with some cases of osteomalacia. Three instances of undoubted general parathyroid enlargement in bitches in association with lactation have come under my observation.

In the reports of partial removal and of transplantation of mammalian parathyroids, particularly in dogs, one of the most characteristic features has been the absence of any noteworthy compensatory enlargement of the remaining portion within the time limits in which other tissues, like the thyroid, heart muscle, kidney, etc., react to artificially induced insufficiencies.

In the course of some experiments with the thyroid gland in fowls in 1910, I observed several instances of marked enlargement of the parathyroids independent of the changes occurring in the thyroid glands. These parathyroid changes were found in fowls which had been fed with maize and wheat for periods of 2 to 6 months. The observations were repeated in 1911, 1912, and 1913, with similar results.

Since calcium temporarily relieves the symptoms of parathyroid tetany in mammals, and since maize and wheat contain very little calcium, it was thought possibly the parathyroid overgrowth might be a result of a calcium deficiency, and if this was so, calcium might exert some protective action against parathyroid overgrowth.

Calcium hydroxide, calcium lactate, calcium carbonate (as chalk and crushed oyster shells), magnesium carbonate, strontium

[1] Erdheim, J., "Ueber Epithelkörperbefunde bei Osteomalacie," *Sitz. Ber. Akad. Wiss.*, 1907, Bd. CXVI, 311–370.

[2] Bauer, T., "Morphologische Studien über die Beziehungen der Epithelkörperchen zum Kalkstoffwechsel," *Frankfurt. Zeitschr. f. Path.*, 1911, VII, 23.

[3] Strada, F., "Le paratiroide nell' osteomalacia e nell' osteoporosi senile," *Pathologica Anno*, I, 1909, 423–437.

carbonate, sodium citrate, sulphuric acid, neutral sulphur and sodium hydroxide have been given in the diet of maize and wheat for periods of one, two and three months. One hundred and ten fowls have been used. No detectable inhibition of the parathyroid overgrowth could be detected in the fowls given magnesium carbonate, strontium carbonate, sulphuric acid, neutral sulphur, sodium citrate or sodium hydroxide,—the growth being as marked as in the controls. On the other hand, those given sulphuric acid and neutral sulphur had more marked parathyroid enlargements and softening of the bones than those given the other chemical substances or the controls. In those fowls which had received calcium there was uniformly less parathyroid overgrowth; in those given calcium hydroxide and calcium carbonate it was barely detectable; while in those given calcium lactate there was moderate enlargement. No differences ascribable to sex could be determined.

These observations suggest: (*a*) that the parathyroids of birds are more susceptible to overgrowth than those of mammals; (*b*) that calcium offers some protection against overgrowth; and (*c*) that the parathyroids (as MacCallum has suggested) are intimately associated with the function of calcium in the complex of body metabolism and nutrition.

74 (891)

Liver necroses associated with Streptococcus infection.

By OSKAR KLOTZ and MAY E. BOTHWELL.

[*From the Pathological Laboratories, University of Pittsburgh, Pittsburgh, Pa.*]

In a series of experiments upon rabbits to determine the tissue reactions to the infection by the *Streptococcus viridans* and having special reference to the heart, arteries, and kidneys, several sporadic examples of necrosis of the liver were encountered. Living cultures of *Streptococcus fecalis*, *Streptococcus mitis*, and *Streptococcus salivarius* were used. Repeated inoculations, from three to five, had been made at intervals of four days.

The earliest necroses appeared in eleven days and consisted

of small focal areas in the peripheral and mid-zones of the liver lobules. In them only a few cells appeared to be affected and seemed to be sporadically picked out in the midst of the liver column. Debris or the ghosts of cells, was all that remained. There appeared to be some edema in the involved area but evidence of thrombosis in the neighboring sinuses was not always demonstrable. In some instances a granular thrombus with fibrin threads was present immediately about the lesion, and at times, extended towards the central vein. Similar thrombi, however, were also observed in areas not showing necrosis.

Some liver columns appeared to show change antecedent to necrosis. In them the cells showed a diminution of nuclear staining with an eosinophile character of the protoplasm. In the vicinity of these again, thromboses were wanting.

Other areas again showed much more advanced necrosis involving not only focal areas but entire lobules or even several neighboring lobules. In all of these instances the necrosis involved the central and mid-zone, while some liver columns still persisted in the vicinity of the portal sheath. In these larger areas thromboses of the mixed fibrinous variety were common. The sinuses of the affected areas were irregularly involved, but not constantly, the central vein being most commonly plugged. These thromboses extended into the sub-lobular vein. Thrombi of agglutinated red blood cells were not observed. There was no inflammatory reaction in the large areas of necrosis nor was there any attempt at restitution either by connective tissue or liver cells.

In 1906, Pease and Pearce[1] noted the occurrence of liver necroses in horses, immunized against the streptococcus pyogenes. In their cases the liver showed diffuse necrosis but they were unable to demonstrate the nature of the process. Since then much literature has appeared in the discussion of liver necroses, and the condition has been described in a great variety of intoxications.

In the absence of thrombi and a cellular reaction in many of the early necroses observed in our cases, it would appear that they have resulted by a direct intoxication by these streptococci. The mixed fibrinous thrombi, developing in the blood channels distal to the liver involvement, probably result from ferments

[1] *Jour. Inf. Diseases*, 1906, III, p. 619.

liberated from the damaged liver cells. Such progressive thrombosis assists in producing a more widespread necrosis of the partly damaged liver tissue, even involving several neighboring lobules.

75 (892)

The effect of gentian violet on protozoa and on growing adult tissue.

By JOHN W. CHURCHMAN, M.D., and D. G. RUSSELL.

[*From the Laboratory of Surgery, Yale University.*]

This study was primarily undertaken to settle two questions raised by the observations made during the last two years on the effect of gentian violet on bacteria.[1] It was noticed early in the gentian violet studies that motile organisms not killed by the stain (violet negative organisms) retained their motility even though deeply stained; and that these stained violet negative organisms when transplanted to agar slants grew equally well with the control smears of unstained bacteria. The retention of motility by the stained organisms might in these experiments be explained as a survival phenomenon; and the growth of transplants made from the stained specimens might be regarded as arising, not from the organisms in the smear which had taken the stain, but from the few in the smear which had escaped it. It seemed altogether likely, from other observations that these explanations were not the correct ones; and that the violet negative organisms actually took the stain during life. Still, definite proof was lacking that gentian violet in these experiments was acting as a true intra-vital stain. To furnish this proof and to investigate the further problem (raised but not solved by the experiments with bacteria) as to whether the vital dye stained the nucleus or the protoplasm, two series of experiments have been done; one with a protozoon (paramecium) and another with living tissues.

EFFECT OF GENTIAN VIOLET ON PROTOZOA.

The paramecium used for this purpose came from a pedigreed race kindly furnished by Professor Woodruff. The effect of the

[1] Churchman, *Journ. Exp. Med.*, Vol. XVI, No. 2, 1912; Vol. XVI, No. 6, 1912; Vol. XVII, No. 4, 1913.

dye was investigated in two ways: 1st, by applying it directly to the organism and transplanting to another media and, 2d, by growing the organism in media containing the dye. In the first series of experiments the paramecium was found to be readily stained and promptly killed. When placed in media containing gentian violet, the effect of the dye could readily be studied on a single organism placed in a watch glass for this purpose. To the fluid in which the organism was swimming gentian violet in a dilution of 1 to 100,000 was added. The effect of the dye was sharp and constant. The nucleus soon became distinctly stained and the cell outline sharply marked out by the staining of the protoplasm. This took place while active motility of the organism was still retained (both rotatory and progressive) and while the cilia were still whipping violently. Before long, however, motility diminished and finally came to a standstill. The cilia continued for some time to wave. After a further interval the movement of the cilia stopped and the organism gradually swelled. Then the cell membrane ruptured, allowing the protoplasm to escape; and the organism either appeared as a deeply stained, motionless and structureless mass, or else persisted only as unrecognizable debris. The effect of the dye was, of course, varied by varying the dilution used. In strengths of 1 to 100,000, cessation of motility occurred in a few moments. In 1 to 500,000, some of the organisms were still motile 48 hours after immersion, but none of them survived. In strengths of 1 to 1,000,000, fission was always delayed and usually prevented, though some of the organisms reproduced slowly, and one which was thought to be faintly stained was seen in the dividing stage.

There was no question that the nucleus of the paramecium was in these experiments deeply stained, while both the organism and its cilia were still actively motile; and, if motility be regarded as a certain indication of life, the observations warrant the conclusion that gentian violet is a true, vital, nuclear stain. We were unable, however, to observe cell division in a *definitely* stained organism; and the motility in these experiments might well be interpreted as a survival phenomenon in a dead or dying organism. The nature of the experiments was, therefore, changed and the effect of the dye on growing animal tissue investigated.

EFFECT OF GENTIAN VIOLET ON GROWING ADULT TISSUE.

For this purpose transplants of pericardium from the adult frog were used; and the media employed was frog's plasma, obtained after the usual technique (Harrison, 1910). The effect of the gentian violet was studied by adding it to the plasma into which the transplants were made. In the first experiments the plasma contained gentian violet in a dilution of 1 to 2,000. Definite growth of tissue occurred in this dilution; but there seemed to be some retardation of growth, and in all subsequent experiments a weaker dilution of the dye was used (1 to 20,000). Here growth was active, keeping pace with the controls. Indeed, in some series the growth in stained media definitely outstripped the controls, and the possibility of a stimulation of tissue growth by weak solutions of the dye should be borne in mind. This growth occurred in spite of the fact that the tissue plant when placed in plasma containing dye became intensely stained.

Our impression after careful study of these growing transplants is that the cell nucleus itself is stained and that this nuclear staining is intra-vital and does not interfere with growth. This much is certain, that in those transplants resting in stained plasma the cell nuclei stand out with great clearness, a clearness by no means to be observed in the controls, and that these clearly seen nuclei appear violet. It is also certain that the cell outline, even when weak dilutions of the stain were used, was rendered very distinct; and in the experiments made with stronger dilutions the protoplasm was definitely stained.

In these observations we paid particular attention to the endothelial cells which grew out in definite sheets. Many of the specimens gave us the impression that the dye might be exercising a selective action; for when the tissue was grown in stained media the new growth took the form of a definite sheet of endothelial cells, not observed when the tissue was grown in unstained plasma. Further experiments are now under way to determine what is the explanation of this undoubted fact.

We have been able to follow the cell division of these stained cells and to observe the whole process of cell division, though the karyokinetic figures have not been clearly seen. We have also observed actively moving cilia in newly produced ciliated endo-

thelial cells from the peritoneum of the female frog, which had been lying in stained plasma for five days.

The growing cells seemed to have the power of gradually changing the stain so that its color fades. That a similar change takes place in the animal body was pointed out in an earlier communication.[1] It was there shown that large amounts of gentian violet injected into the ear vein of the rabbit soon disappeared from the blood and that the mucosa of the tongue and lips, though at first deeply stained, in a short time (about 48 hours) lost their violet color. Animals killed a few days after the injection showed no trace of the dye, nor did it appear in the urine. Attention was called in this paper to the care which must be used in speaking of the non-toxicity of this and other dyes; for, as was there suggested, the tolerance of the dye by the animal might have been due to the animal's ability to change the substance into another actually non-toxic one.

In the original communication on the selective bactericidal action of gentian violet the suggestion was made that similar studies should be carried out on the effect of the dye on growing tissue. This communication contains the first report of work undertaken in that direction, which is still being prosecuted in this laboratory. We have also under way an investigation of the effect of gentian violet and other dyes on growing embryonic tissue from the tadpole. The significance of the results thus far obtained seems to us to be:

1. The successful growth of tissue in stain-containing media suggests the possibility that stains may be found which have a selective action on tissue, similar to that possessed by gentian violet for bacteria; and that in this way pure cultures of tissue may be possible out of mixtures, just as pure cultures of bacteria may now be so obtained.

2. Certain animal tissues grow readily in gentian violet of a far stronger dilution than that necessary to kill many contaminating bacteria. In these experiments, for example, successful tissue growths were obtained when gentian violet, 1 to 20,000, was used; yet bacillus subtilis will not grow in 1 to 100,000 and grows very badly in 1 to 1,000,000. This fact may simplify the technique of tissue growth by eliminating the risk of bacterial contamination.

[1] Churchman and Herz, *Journ. Exp. Med.*, Vol. XVIII, No. 5, 1913.

3. If our impression that the nucleus of the growing cell is actually stained prove correct, the use of stains in the plasma in which tissue is grown should certainly facilitate the study of nuclear growth.

4. Certain observations made last year in this laboratory (too few to serve as more than a suggestion) seem to indicate that another dye (methylene blue) acted as a stimulant to the growth of connective tissue. This lead also should be followed out and the effect of all possible stains studied in the hope of discovering dyes which will have a sharp selective action on growing tissue.

5. The growth of animal cells in a strength of dye much more than sufficient to kill many pathogenic organisms lends encouragement to the efforts now being made in this laboratory to apply the observations on the bactericidal effect of gentian violet and allied stains to the treatment of disease.

76 (893)

On the hexosamine of chondroitin sulphuric acid.

By P. A. LEVENE and F. B. LA FORGE.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*]

In a previous communication[1] the writers reached the conclusion that the nitrogenous component of chondroitin sulphuric acid was glucosamine. The conclusion was based on the analytical data of the hydrochloride of the amino sugar, and on the magnitude of its optical rotation.

However, recently it was discovered that the optical activity of the amino sugar differed considerably from that of glucosamine, if measured under very definite conditions. The conditions required are the following: low temperature of the solution, comparatively high concentration of the sugar solution, and measuring the initial rotation immediately after the solution of the sugar is accomplished. Under such conditions it was found that the specific rotation of the amino sugar of the chondroitin sulphuric acid was about 25 per cent. higher than that of glucosamine. Both

[1] *Jour. Biol. Chem.*, XV, p. 155, 1913.

substances displayed mutirotation and reached equilibrium simultaneously. Also in its melting point and in its solubility the amino sugar differed from glucosamine. Finally the osazones of the two substances differed in their melting points and in their solubility in alcohol and in water.

On the basis of these data it seems justified to conclude that the hexosamine is not identical with glucosamine, but is isomeric to it.

Work on the configuration of the sugar is in progress.

Substance.	Crystal-form.	Rotation. Initial. $[\alpha]_D^{0°}=$	Rotation. Final. $[\alpha]_D^{0°}=$	Solubility.	Melting Point.
Glucosamine hydrochloride.....	Short thick prisms.	+101.6	+73.65	Difficultly sol. in 80 per cent. EtOH; insol. in abs. EtOH.	Decomposes slowly above 200°.
Osazone.....	Needles.			Difficultly sol. in abs. EtOH.	Melts with decomposition 206°.
Hexosamine hydrochloride.....	Long prismatic needles.	+129.5	+93.82	Easily sol. in 80 per cent. EtOH; difficultly sol. in abs. EtOH.	Melts with decomposition 180°
Osazone.....	Long needles.			Easily sol. in EtOH; sol. in hot H_2O.	175–180°

ABSTRACTS OF COMMUNICATIONS, PACIFIC COAST BRANCH.

Fifth Meeting.

San Francisco, California, April 9, 1914.

77 (894)

The pressor compounds of the pituitary gland.

By ALBERT C. CRAWFORD and ZENO OSTENBERG.

[*From the Division of Pharmacology, Stanford University Medical School.*]

The pituitary gland of cattle contains several pressor principles, one or more of which dialyze and at least one which does not dialyse through parchment paper. The non-dialyzable compound or compounds can be purified by lead subacetate and the filtrate, freed from lead, does not give the biuret reaction, at least in dilutions which give a pressor reaction, but yields a white benzoate (Baumann-Schotten method). The depressor principle readily dialyzes, but apparently depressor compounds form on long standing of the non-dialyzable portion.

78 (895)

Note on the action of epinephrin on the guinea-pig uterus.

By E. BARBARA WILL and ALBERT C. CRAWFORD.

[*From the Division of Pharmacology, Stanford University Medical School.*]

On the isolated horn of the non-pregnant guinea-pig uterus suspended in oxygenated Locke's solution (Dale's modification), epinephrin (0.9 mg. in 500 c.c. at 41° C.) produced relaxation. Longitudinal strips also relaxed. Circular strips gave slight imperfect contractions. Direct inspection of the intact uterus showed that the intravenous injection of epinephrin produced blanching with contraction of the circular fibers. Diminution in the diameter of the horn and elongation was observed. This would suggest that the difference in action of epinephrin on the

non-pregnant and pregnant uteri of some animals may really be due to a relative increase in the development of the circular fibers. On the uterine horn in early pregnancy, epinephrin also caused relaxation. Circular strips contracted. Later in pregnancy the circular fibers gave a more marked response. On longitudinal strips taken from the same horn, epinephrin caused an increased tone with increase in number and strength of what Pettenger calls the "remittent contractions," but a suspension of what is called the intermittent contraction. Of course it is a question whether the so-called "remittent contractions" are not due to the presence of circular fibers. Direct inspection of the pregnant uterus in situ showed that epinephrin caused blanching and constriction of the circular fibers, with probably relaxation of longitudinal fibers. An oxidized solution of epinephrin, without pressor action, made by passing air through a solution of epinephrin for two days, caused marked increase in frequency of contractions. Experiments on the longitudinal fibers (during late pregnancy) are as yet incomplete, but would indicate that epinephrin caused relaxation.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Fifty-ninth meeting.

Zoölogical Laboratory, Columbia University, May 20, 1914.
President Lusk in the chair.

79 (896)

A comparison of the effects of labyrinthine and cerebellar lesions in the turtle.

By J. Gordon Wilson and F. H. Pike.

[*From the Otological Laboratory of Northwestern University and the Physiological Laboratory of Columbia University.*]

It is of some interest, in view of the tendency in some quarters to insist upon the essential similarity of the effects of lesions of the labyrinth and of the cerebellum, to ascertain whether this supposed constancy of relationship obtains in animals with a relatively small cerebellum. It may be mentioned that, anatomically, the floor of the mid brain (the pons) and that portion of the roof represented by the vermis cerebelli are phylogenetically old. The lateral lobes of the cerebellum are new developments. The cerebellum of turtles is represented by the vermis. The cerebellum in the genera of turtles used for experiment—*Nanemys* and *Chrysemys*—is smaller than in the sea turtles.

We have already pointed out the fact that there is a great uniformity in the effects of labyrinthine lesions in all animals so far studied[1] and that the effects in the turtle are much the same as in other animals.[2]

[1] Wilson and Pike, *Philosophical Transactions of the Royal Society of London*, 1912, Series B, Vol. 203, p. 157.

[2] Wilson and Pike, Proceedings of the Society for Experimental Biology and Medicine, 1913, XI, p. 52.

Ablation of the cerebellum in the turtle does not give rise to the same train of symptoms that is observed after labyrinthine extirpation. We find, in agreement with Fano,[1] that the righting reaction is not abolished by ablation of the cerebellum. There was a small, scarcely noticeable, disturbance of coördination in swimming, manifested most clearly in a slight awkardness in approaching the side of the tank. Occasionally, lack of precision of limb movements on the injured side was observed after unilateral operations. One other symptom was that the animal was rarely or never seen to swim deep in the water after cerebellar removal. Our observations substantiate those of Fano, Bickel[2] and Sergi,[3] whose experiments were done on different genera of turtles.

The results on turtles are in substantial agreement also with those of Steiner,[4] Loeb,[5] Bethe[6] and Corso,[7] who report no noticeable motor disturbances in sharks (*Scyllium*) after cerebellar ablation.

It is clear that, in certain of the lower vertebrates, there is a constant and striking difference between the effects of labyrinthine and of cerebellar lesions, and it is equally clear that in these forms the cerebellar connections of the labyrinth are not the important connections.[8]

80 (897)

The involvement of the blood and blood vessels in infantile scurvy.

By ALFRED F. HESS.

[*From the Research Laboratory, Board of Health, N. Y. City.*]

Infantile scurvy is a disease characterized by malnutrition, and a tendency to bleeding, especially in the gums, and under the

[1] Fano, *Archives italiennes de Biologie*, 1883, III, pp. 365–368.

[2] Bickel, *Archiv für [Anatomie und] Physiologie*, 1901, pp. 52–80; pp. 495–498.

[3] Sergi, *Arch. di Farmac. sper. e Sc. affin.*, 1905, IV, pp. 474–515.

[4] Steiner, Die Funktionen des Zentralnervensystems und ihre Phylogenese. II. Die Fische; Braunschweig, 1888.

[5] Loeb, *Archiv für die gesammte Physiologie*, 1891, L, pp. 68–85.

[6] Bethe, ibid., 1899, LXXVI, pp. 470–493.

[7] Corso, *Archives italiennes de Biologie*, 1895, XXII, p. xciv.

[8] Luciani, "Physiologie des Menschen," 1907, Bd. III, pp. 482–489.

periosteum of the long bones. It is very often classed under the hemorrhagic diatheses. There has been no comprehensive study of the blood made in this condition. I have had an opportunity to examine the blood in eight cases, more specially from the point of view of coagulation. As is well known there is more or less secondary anemia, a deficiency of hemoglobin and of red blood cells. In addition to this there has been in all the cases examined an increase of leucocytes. The platelets which have been examined several times in each case have been found to be normal in number.

Special attention was paid to the coagulability of the blood. For this purpose the blood was removed directly from the veins into sodium oxalate, was centrifuged, and the plasma was titrated with varying amounts of ½ per cent. calcium chloride solution. At least two tests of this nature were made in each case. The prothrombin was found to be about normal. In a few cases it was somewhat less than that of the normal control, which was always tested at the same time and in the same way as the case of scurvy. The antithrombin was not found to be in excess. The calcium, which by some has been blamed for the bleedings, was found to be quite sufficient for coagulation. That is to say, in the prothrombin test no more calcium had to be added to produce a clot in the cases of scurvy than in the normal cases.

Having found that the blood was normal in these respects, a test of the blood vessels was carried out. For this purpose a method was used which may be termed the *capillary resistance test*—the large vein was constricted in the upper arm by a blood pressure apparatus, using a pressure of about 80 mm. of mercury, and this pressure was continued for exactly three minutes. The bandage was then removed and petechial spots were looked for upon the forearm. Normally this degree of pressure is insufficient to bring about petechiæ. In the case of scurvy, however, numerous little hemorrhages were found to follow this compression of the veins. This result is met with regularly. It may be argued from this result that the blood vessels in this disease suffer with the tissues in general, and that as a result they become more permeable. This is probably not distinctive of scurvy, but occurs in other diseases where the capillaries are involved. It may be

added, however, that tests on several cases of hemophilia have shown that the blood vessels are not affected in this disease, and that the blood does not permeate the vessel wall when subjected to this amount of increased pressure.

81 (898)

A note on the retention in the blood of uric acid and creatinine in the uremic type of nephritis.[1]

By V. C. MYERS and M. S. FINE.

[*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.*]

In two cases of nephritis of the uremic type with high non-protein nitrogen and urea, very high figures for both uric acid and creatinine have been observed. The increase in the concentration of these substances is best shown in tabular form. The figures are in mgm. per 100 c.c. of blood.

Case.	Date.	Non-protein N.	Urea N.	Uric Acid.	Creatinine.	Creatine and Creatinine.
1	Mar. 7	292	200	10.5	....	....
				11.0	9.0	15.0
	Mar. 9	207	182	11.4	16.6	....
2	May 4	...	...	6.1	....	....
	May 7	155	120	8.0	10.0	16.0
	May 13 a.m.	184	140	13.7	13.9	17.2
	May 13 p.m.	226	170	14.0	14.7	27.8

The progressive increase in the various constituents as the condition approaches a fatal termination is well shown in Case 2. Attention is called to the possible etiological importance of the retention of creatinine on account of its containing the toxic guanidine group, also to the probable diagnostic and prognostic value of the determinations for uric acid and creatinine in this con-

[1] After the title of our communication was submitted, papers appeared by O. Neubauer, *Münch. med. Wochenschr.*, (Apr. 21) 1914; LXI, p. 857, and by Folin and Denis, *Journ. Biol. Chem.*, (May) 1914, XVII, p. 487, reporting somewhat similar observations. Neubauer records one case of uremia with marked retention of creatinine in the blood, while Folin and Denis present ten cases of uremia with analyses of uric acid, creatinine together with the other nitrogenous constituents.

dition. The possible etiological bearing of the retention of creatinine is being considered from the standpoint of experimental nephritis.

Estimations of total solids, total nitrogen, chlorides, sugar, and cholesterol have been made in all our cases in addition to the above determinations. In our earlier estimations of creatinine, Shaffer's suggestions for the estimation of creatinine in dilute solutions were followed, but recently we have employed Folin's new method which has been found very satisfactory.

82 (899)

The significance of the non-protein nitrogen of the blood in experimental uranium nephritis.

By HERMAN O. MOSENTHAL.

[*From the Department of Medicine and the Laboratory of Biochemistry, Columbia University.*]

The increase in the non-protein nitrogen content of the blood in the experimental uranium nephritis of dogs may be due to:

1. *Diminished secretory activity of the kidney.* This is undoubtedly the case in those instances of severe nephritis in which the urinary nitrogen is diminished.

2. *Increased protein catabolism.* In poisonings of less intensity but sufficient to produce a nephritis of considerable severity as indicated by the albuminuria, there may be an increased amount of nitrogen in the urine as compared to the intake. In these instances the amount of non-protein nitrogen in the blood rises considerably. Such an increase, not being due to nitrogen retention on the part of the kidney, may be ascribed to an increased protein catabolism.

3. *Inspissation of the blood.* A polyuria, resulting in loss of water to the animal in this form of nephritis, may cause an apparent rise in the non-protein nitrogen of the blood.

4. *The chemical combination in which the non-protein nitrogen of the blood exists.* Animals with a certain degree of uranium nephritis are capable of putting out extremely high amounts of nitrogen in the urine. (A dog of 15 kilos daily eliminated 23 gm.

of nitrogen, half of which was given as urea, throughout a uranium nephritis without any retention.) This would seem to indicate that the increase in non-protein nitrogen of the blood in such animals is due in part to an abnormal chemical combination which can not pass the kidney and is not necessarily due to impaired kidney function.

Before ascribing an increase in the non-protein nitrogen of the blood in any form of nephritis to kidney insufficiency, the influence of all the above factors should be taken into account.

83 (900)

The influence of induced diabetes on malignant tumors (including a report of a case of human phlorhizin glycosuria).

By STANLEY R. BENEDICT and ROBERT C. LEWIS.

[*From the Department of Chemistry, Cornell University Medical College, and from the Research Laboratory of General Memorial Hospital, New York City.*]

Experiments have been reported by Beebe and Van Alstyne showing that withdrawal of carbohydrates from the diet of white rats markedly inhibits the "taking" and rate of growth of transplantable malignant tumors in these animals. Cremer and Lockhead have recently shown that carbohydrate is utilized in the growth of tumor tissue in experimental tumor rats. As a development of the idea that utilization of glucose plays an important rôle in the synthesis of new protoplasm, we have carried out experiments upon rats planted with the "Buffalo sarcoma" in which the animals were placed upon a carbohydrate-free diet, and at the same time rendered diabetic through the injection of 0.2 gram doses of phlorhizin in olive oil once in two or three days.

As a brief summary of our results in this connection it may be stated that we have worked with about forty rats in all, so far, and that the results are so striking and constant as to warrant a positive statement that the production of the complete diabetes in experimental sarcoma rats is followed by retrogression and ultimate cure of the growth in every case where the growth at the

time of beginning of the treatment does not exceed 20 by 25 millimeters, and the animal will survive the treatment for a period of at least ten days. In every case where the animal lives for three days a marked effect upon the growth is to be noted. Small growths show no local reaction, but begin to retrogress at once, while in very large growths there is rapid development of local inflammation and softening. Small growths go on to complete cure, while with large growths the death of the animal most frequently occurs before much of the growth has been absorbed.

The following protocol of a single experiment is given as illustrative. On May 10, 1912, the growth measured 12 × 10 mm., on May 11, 14 × 11 mm., on May 13, 16.5 × 13 mm., on the 14th, 19 × 14 mm. The treatment was begun on May 14, and continued for twelve days. On May 16 the growth measured 16 × 12 mm., on the 18th 14 × 11 mm., on the 21st, 10 × 8 mm., on the 25th, 4 × 3 mm. On June 10 no trace of the growth could be felt on or under the skin. Early in July the animal was chloroformed. Autopsy showed no detectable growth anywhere. This experiment is one of a number of practically similar ones. Controls have always been employed, and many experiments have been carried out upon animals where the growth was unquestionably past the stage where spontaneous retrogression would occur. The largest growth which we have succeeded in curing by the above indicated treatment measured 45 × 47 mm. We believe that this is by far the largest experimental malignant growth which has so far been successfully treated.

Upon the basis of the above summarized results, we were led to apply a similar method of treatment to some human cases of cancer at the General Memorial Hospital, under the supervision of Dr. James Ewing and Dr. Richard Weil. In some cases the results were slightly encouraging, but the results obtained with rats were far from duplicated. We have so far applied the method in only a very few desperate cases of human cancer, and we believe that the treatment may still be worked out so as to be of value as a therapeutic measure.

We have studied the glycosuria following injection of phlorhizin and withdrawal of carbohydrate from the diet in one case in detail and may summarize our findings by stating that the urine picture

exactly duplicates in all essential features the urinary findings in the later stages of severe types of diabetes mellitus. The dextrose to nitrogen ratio is approximately 3.6 upon a carbohydrate free diet. Glucose given at this time was quantitatively recovered. In spite of doses of sodium bicarbonate ranging from 20 to 30 grams per day the ammonia output rose to over four grams per day, representing 27 per cent. of the total nitrogen at its highest level. Oxy-butyric acid, diacetic acid, and acetone were eliminated in large amounts. When large quantities of glucose were given the urine picture returned to normal almost at once, and the diabetes completely ceased when phlorhizin administration was stopped, the patient rapidly returning to normal condition. Considering the severity of the treatment the patients stand it surprisingly well. They lose weight, but the general condition does not at all duplicate the later stages of diabetes mellitus.

The findings in this case will be reported in detail elsewhere.

84 (901)

A study of further generations of mammals from ancestors treated with alcohol.

By CHARLES R. STOCKARD.

[*From the Department of Anatomy, Cornell Medical School, New York City.*]

Experiments now in progress for almost four years have demonstrated the fact that the germ cells of male guinea-pigs can be so injured by allowing the animals to inhale the fumes of alcohol that they give rise to defective offspring although mated with vigorous untreated females.[1]

In the present communication I wish to emphasize the fact that the effect of this injury of the germ cells is not only shown by the immediate offspring of alcoholized animals but is conveyed through their descendants for at least three generations.

The offspring from the treated guinea-pigs which reach ma-

[1] *Archiv f. Entw.-Mech.*, XXXV, 1912; *Archives of Int. Med.*, X, 1912; *American Nat.*, XLVII, 1913.

turity are usually nervous and slightly under sized. These animals F_1, or the second generation, are never themselves subjected to the fume treatment. Mated even with normal animals the results are poor when compared with the outcome of normal control matings. Twenty-six matings of second generation with normal animals gave in 4 cases negative results or early abortions, 2 stillborn litters of 4 individuals and 20 living litters containing 31 young, 19 of which died and only 12 survived. Twenty-two matings of second generation by alcoholized animals gave 5 negative results or early abortions, 3 stillborn litters of 7 young and only 14 living litters consisting of 25 young, 11 of which died and 14 survived.

If second generation animals, non-relatives in most cases, are mated with one another the sum total of the result is worse than when they are mated in either of the above combinations. Forty-seven such matings gave in 14 cases negative results or early abortions, 3 stillborn litters containing 8 young, and only 30, less than 64 per cent., living litters consisting of 46 young; 14, or about 33 per cent., died soon after birth and 32 survived.

A point of interest is that several of these offspring, F_2, or third generation, show gross defects or deformities. Only two of the immediate offspring from alcoholized animals have shown a clouded condition of the cornea of one eye, and no defect or deformity of any nature has been observed in 89 control young. However, among the 54 F_2, or third generation, young that have reached term 9 or about 17 per cent. show gross eye defects. Two had opaque corneas, 3 complete cataracts in both eyes, the lens being milk white, 2 had one normal eye while the other was about half size and blind, one has only one eye, that of the opposite side being completely absent, and finally one animal was entirely eyeless, having no indication of eyeballs, optic nerves or chiasma.

These abnormal animals arose from parents that had not been subjected to the alcohol treatment, although in all cases two or more of their grandparents, and usually only the paternal ones had been treated for various lengths of time with the fumes of alcohol.

Only a few matings of the third generation, F_2, animals have been made, yet if conclusions may be based upon these small

numbers the outcome is more unfavorable than from second generation matings. Six such matings gave in 2 cases negative results or early abortions, 1 litter of 2 stillborn young and 3 living litters containing 5 animals, only 1 of which survived, 4 dying soon after birth.

Two of the four were completely eyeless, the eyeballs, optic nerves, and chiasma being absent. There was an abortive attempt at eyelid formation, a well-formed lachrymal gland and the extrinsic eye muscles were present. These defects no doubt result from injury inflicted upon the germ cells by the experimental treatment. The parents of the anophthalmic guinea-pigs just mentioned were untreated, the four grandparents were also untreated but their great-grandfathers were all alcoholized and their great-grandmothers were all normal animals. It thus appears that the injury received by the germ cells of the great-grandfathers was responsible for the defective condition of their descendants. Many of the defective young have normal maternal ancestry and alcoholized paternal ancestors, the reverse being also true.

Although the descendants of alcoholized males seem to transmit the defects through subsequent generations even more decidedly than the offspring of treated females, yet it is peculiar to find that in pairing F_1, second generation, animals with normal mates if the male of the pair be F_1 the resulting offspring are better and more vigorous than when an F_1 female is mated with a normal male. Eleven matings of F_1 males with normal females gave 1 negative result, 1 stillborn litter of 2 young and 9 living litters of 11 young, 9 of which survived but later gave rise to defective descendants. Fifteen matings of F_1 females with normal males gave 3 negative results, 1 stillborn litter of 2 young and 11 living litters containing 20 young, only 3 of which survived; a result many times more disastrous than that derived from the previous combination.

Since the direct action of the alcohol fumes on the cornea of the eye finally renders many of the treated animals blind one might imagine some connection between this and the defective eye condition of the offspring and their descendants, yet such is certainly not the case. The defective eyes of the descendants

of treated animals are due to a generally weakened or impaired development. The male germ cells are weakened or injured by the alcohol treatment and all individuals arising from combinations involving such a germ cell are below normal. In this connection Cole and Davis[2] have recently recorded an interesting experiment with rabbits. They sought to control the experiments on the effects of alcoholic inhalations in a very ingenious way. "By breeding a male homozygous for color and an albino male both to an albino female it is possible to assign the young to their respective fathers, since the offspring of the colored male will be colored and those of the albino male will be albinos. If one of the males now be alcoholized while the other is normal, and offspring from both result, any differences, such as defects in the offspring, may safely be attributed to the effects of the alcoholizing of the male, since both sets of fetuses have developed in the same uterus at the same time, and consequently there can be no question of different environmental influences."

A preliminary test of 36 double matings was made in which both males were normal. One pigmented male was used in 23 of these matings, an albino male also being used in each case—190 offspring were produced and the albino male sired only 24 of them, 166 coming from the pigmented sire. This showed a strong individual potency for the colored male. Yet after he had been alcoholized, by the inhalation method, *he failed to sire any offspring at all* when used in conjunction with an albino male, although he was bred to the female first in at least 5 of the 7 matings made. When bred alone to normal females he sired several litters of young which later showed certain indications of defects. These experiments demonstrate conclusively that the spermatozoön is actually weakened or disabled by the alcohol treatment, as I had formerly concluded in explaining the defective offspring from alcoholized male guinea-pigs.

[2] *Science*, N. S., XXXIX, 1914.

85 (902)

The action of radium on growing cells.

By F. C. WOOD and FREDERICK PRIME.

[*From the Crocker Laboratory for Cancer Research,* Columbia University.*]

If it is difficult to correlate the published results of the clinical treatment of cancer with radium, it is still more difficult to correlate the findings in the biological study of radioactivity. My own experiments on primary mouse tumors, shortly to be published, show that it is impossible to cure primary carcinoma of mice by the application of 155 mgm. of radium bromide, even when used for long periods of time. Nevertheless, the tumors so treated shrink to a fraction of their previous size. Thus, after forty-eight hours' treatment of a tumor, about 15 mm. in diameter, with 30 mgm. of radium bromide, only a microscopic remnant could be found. von Wassermann[1] says, however, that the direct application of 55 mgm. of mesothorium for many days did not interfere with the growth of a transplanted mouse carcinoma, and yet he states that a small fragment of the same tumor irradiated for three hours with the same amount of mesothorium could not be successfully transplanted. Again, he says that carcinoma cells suspended in Ringer's solution have their "genoceptors" destroyed so that the cell cannot reproduce itself, though it is still alive and its nutrireceptors are active after three to three and a half hours. The method he uses to prove that the cells are still living is to suspend them in methylene blue solution; if this decolorizes, he considers that the cells are alive. The assumption is so questionable that it seems worth while to publish a few experiments out of a large series made in the Crocker Laboratory as a part of a general study of radium action.

Russell and Bullock[2] have recently taken issue with Von Wassermann and have drawn attention to some observations which contradict his statements, citing the experiments of Russ and

* George Crocker Special Research Fund.

[1] *Deutsch. med. Wchnschr.*, 1914, p. 524.

[2] *Berl. klin. Wchnschr.*, 1914, p. 725.

Wedd,[1] who discovered mitoses six days after the irradiated grafts had been implanted.

The preliminary experiments now to be reported do not wholly clear up these conflicting statements, and it is certain that a great deal more work must be done on a great variety of tissues with carefully measured quantities of radium and standard screening before we can obtain any insight into the effects exerted by this substance. We have noted, however, that 155 mgm. of radium bromide, screened with 1 mm. of aluminum and 0.18 mm. of coverglass, did not stop the beating of embryonal heart tissue in vitro, nor check a profuse outgrowth of connective tissue from the mass, after an exposure of three hours. So, too, the Flexner rat carcinoma, growing in rat plasma, when treated with 155 mgm. of radium bromide, screened with 0.4 mm. brass and 0.18 mm. coverglass, was not entirely inhibited in its growth by a three hours' exposure, though the amount of radiation was three times that used by von Wassermann.

In another series of the same tumor, however, growth was inhibited after an exposure of three hours to 155 mgm. of radium bromide, screened with 0.8 mm. of brass and 0.18 mm. of coverglass

These absolutely contradictory results, representing a considerable series of experiments, show how cautiously we must draw conclusions as to the action of radium on cells when they are placed in unfavorable surroundings.

The growth of the Jensen rat sarcoma was inhibited, but not stopped, by an exposure of three hours to 30 mgm. of radium bromide, screened with 0.8 mm. of brass and 0.18 mm. of coverglass, while an exposure of three hours to 155 mgm. with the same filter stopped all growth. In this case, apparently, the sarcoma was more susceptible to radium than the carcinoma, while embryonal tissue was the most resistant of all. Similar differences were noted by Menten in radiating transplanted tumors. The same tumor tissue in vivo when exposed to radiation of far greater intensity is uninjured, as shown by its transplantability.

In conclusion, then, 155 mgm. of radium bromide, screened with 1 mm. of aluminum or 0.8 mm. of brass and only about 1.5

[1] *Arch. Middlesex Hospital*, 1912, XXVII, 50; see also Russ and Chambers, *ibid.*, 1913, XXX, 120.

mm. distant from beating embryonal heart tissue, does not kill it in three hours, and does not stop the growth of connective tissue cells. The same exposure, however, does prevent the growth of Jensen rat sarcoma, and inhibits but does not wholly prevent the growth of the Flexner rat carcinoma. Observations such as these show the danger of generalizing too freely from a limited number of experiments.

86 (903)

Note on the effect of animal extracts upon the secretion of the pancreas.

By ISAAC OTT, M.D., and JOHN C. Scott, M.D.

[*From the Laboratory of the Medico-Chirurgical College of Philadelphia.*]

Our experiments were made upon etherized cats who were killed before regaining consciousness. A Bernard cannula was inserted into the pancreatic duct inside the lumen of the intestine. The biliary duct was previously ligated. We then injected secretin solution into the jugular, as no secretion was noted before its injection. We then counted, after the injection of secretin, the number of drops falling every 5 minutes for three periods. Then we injected the same amount of secretin plus a watery solution of one of the dried glands. Then we counted for three periods the number of drops every 5 minutes. Finally we again injected the same amount of secretin solution and again noted the number of drops every 5 minutes for three periods. If in the second period we obtained a marked increase over or decrease below the first period and third period, we inferred that the animal extract had some action. We obtained the following results. We also have inserted their effects upon the volume of the gland for comparison.

Animal Extracts.	Pancreatic Volume.	Pancreatic Secretion.
Parathyroid	increases	increases
Secretin	increases	increases
Mammary	increases	increases
Infundibulin	decreases for 3 minutes then increases	decreases (Pemberton & Sweet)
Adrenalin	decreases for 3½ minutes then increases	decreases (Pemberton & Sweet)
Pinea	increases	increases

These experiments were performed before the recent judicial decision in Pennsylvania upon experimentation.

87 (904)

Metabolism studies in a case of congenital hemolytic jaundice with splenomegaly.

By JAMES P. MCKELVY, M.D.,
and
JACOB ROSENBLOOM, M.D., PH.D.

[*From the Laboratory of James P. McKelvy, M.D., Pittsburgh, Pa.*]

In a case of congenital hemolytic jaundice with splenomegaly, we have found in a metabolism experiment of five days, on the Folin diet, a loss of 4.06 grammes of nitrogen, while the urinary nitrogen partition was normal in character, with the exception of the uric acid nitrogen, which was increased. The absorption of nitrogen was normal.

The urinary sulphur partition was normal in character with occasional increased excretions of ethereal sulphates. In the five days, there was a loss of 18.8 grammes of sulphur, 0.482 grammes of calcium oxide and 0.924 grammes of magnesium oxide. There was a phosphorus retention of 0.07 grammes, while the amounts of earthy phosphates and total phosphates may be considered normal.

There was a loss of 0.1199 grammes of iron, with marked increased amounts of iron excreted in the urine and feces. The fat metabolism was normal, with an absorption of about 91 per cent. of the ingested fat. The amounts of neutral fat, fatty acids and soaps in the stool were normal.

A marked disturbance in the cholesterol metabolism was found, and the hypothesis is advanced that a lack of cholesterol in the blood serum may account for the increased hemolysis; and the splenomegaly may play some rôle in the cause of this condition.

Urobilin and urobilinogen were present in the urine and feces, while bilirubin and hemoglobin were absent.

88 (905)

Weight fluctuation in frogs.

By C. C. GUTHRIE and F. V. GUTHRIE (by invitation).

[*From the Physiological Laboratory, University of Pittsburgh.*]

It is known that frogs lose weight when removed from water or very moist places and are placed in dry surroundings; and that gain in weight occurs under reversed conditions.[1] To test the magnitude of such change under local laboratory conditions, beginning in 1909, several series of experiments have been performed. Leopard frogs (*Rana Pipiens*) were used.

The first series were performed during June and July and the laboratory was not heated. During the day the windows were opened and the temperature varied from about 23.6° to 30.2° C. Night temperatures were not taken, but the minimum observed by the local weather bureau for the period was 16.7° C. Showers occurred during the experiment, so the air was not abnormally dry.

Placed in water and weighed hourly, a maximum hourly fluctuation of 10 per cent., a minimum 0, and an average for 10 hours of 3.8 per cent. was observed. A total gain in weight amounting to 20 per cent. occurred. Placed in wire cages in the air of the laboratory a very rapid loss of weight occurred, amounting to as much as 40 to 45 per cent. of the original weight in twenty-four hours. If the loss was not greater than this, recovery might take place if the animal were placed in water. The gain in weight was very rapid, the total weight within four hours amounting to as much as 121 per cent. of the original weight, or 200 per cent. of the weight after drying. During the next sixty hours, the weight fluctuated moderately, rising during the afternoon of the second day to 136 per cent. of the original weight before drying.

The time of death by drying is not very exactly shown by the experiments, owing in part to the sluggish reactions supervening. In one case the animal was alive thirty hours after being placed in the dry cage at which time the loss of weight amounted to 42

[1] Donaldson, *Jr. Compar. Neurol.*, 1898, VIII, 314. A. Durig, *Arch. f. d. ges. Physiol.*, 1901, LXXXV, 401; 1901, LXXXVII, 42; 1902, XCII, 293.

per cent., while seventeen hours later (over night), the animal was dead, and the loss of weight had increased to 56.4 per cent. of the original weight. This is rather larger than loss in weight compatible with life hitherto reported, but as it is known that the seasonal condition of the animals has an important bearing in such experiments,[1] and since the frogs employed were in the "winter" condition, the relatively great loss in weight before death is not considered extraordinary.

A frog in which the brain was pithed before placing in the dry cage lost 60 per cent. of its original weight in twenty-three hours,

The behavior of partially dried frogs when placed in hypo-, iso- and hypertonic salt solutions is interesting. In one such experiment, the original weight was 19 gm. and twenty-three hours after placing in a dry cage 11 gm. The frog was then placed in 0.2 per cent. NaCl solution. Fifty minutes later it weighed 18 gm., and seven hours later 25 gm. Twenty-four hours later the weight was 24 gm., from which point, with slight fluctuations. it gradually decreased until seventy-two hours after placing it in salt solution it was 22 gm. The animal remained lively.

Another frog weighing 18.5 gm. was allowed to dry until it weighed 11.8 gm., which took place within twenty-three hours. It was then placed in 0.6 per cent. NaCl solution. Fifty minutes later it weighed 16 gm., and seven hours later, 22 gm. Fifty-five hours after being placed in the solution, the weight was 28.2 gm., but seventeen hours later it was 24.5 gm. At this time the frog was alive and in good condition.

Another frog similarly dried and placed in 0.7 per cent. NaCl solution, showed a more gradual and less pronounced increase in weight, and was active and in good condition seventy-two hours later. Frogs dried and placed in 0.8 per cent., 0.9 per cent. and 1 per cent. NaCl solutions barely regained their original weight, and the process was much more gradual. They died soon after being placed in the NaCl, but in the case of 1 per cent. NaCl the animal lived for somewhat longer than seven hours. In stronger solutions, body weight was not regained, and death soon occurred.

Since it is known that water content influences tissue function, this note is offered with the view of directing attention to the

[1] Donaldson and Schoemaker, *Jr. of Comparative Neurology*, 1909, X, 109.

fact that under laboratory conditions the weight of frogs may fluctuate rapidly and extensively; and further, to emphasize the importance of adequate precautions to prevent such changes in physiological and pharmacological studies on frogs, especially when it is particularly important to preserve normal physiological reaction in the highest degree or in determining pharmacological dosage in relation to body weight.

89 (906)

The sensory effect of local application of hypertonic salt solutions.

By **C. C. GUTHRIE** and **M. E. LEE** (by invitation).

[*From the Physiological Laboratory, University of Pittsburgh.*]

While engaged in an investigation of certain effects of local application of salt solutions to exposed nerve trunks, and to nerve terminations exposed by abrading the skin, a paper appeared by Wiki,[1] in which results are presented and interpreted as showing that local anesthesia followed intracutaneous injections of solutions of various substances into guinea-pigs. A number of the salts employed by him were included among those used by us. Since the interpretation of an anesthetic action by him is at such variance with our results, it seems advisable to make a brief statement at this time.

Wiki states that strong solutions of magnesium chloride or sulphate when injected intracutaneously in the back of a guinea-pig produce marked anesthesia, as evidenced by decreased reflexes upon stimulating the affected skin area; while in our experiments, direct application of strong solutions of these salts to exposed nerve trunks (frog and turtle), abraded skin areas (human), or to the unabraded skin (frog), have resulted in very positive evidence of an irritant action. In the case of nerve trunks thoroughly isolated from surrounding tissue, a block may be produced by keeping the nerve bathed in a strong solution; but in the case of application to abraded or unabraded skin, though such applications have been continuously applied for twenty minutes or more, not one symptom of anesthetic action could be

[1] *Jr. de Phy. et Path. Gen.*, 1913, XV, 845.

detected. A number of other solutions and substances were tested in the same way and all, with the exception of cocain, gave similar results, the only difference being either a difference in the time of onset of evidence of irritant action or in the degree of such action.

Among the substances investigated by application to abraded skin areas, magnesium salts were among those that gave rise to very severely painful sensations which persisted for twenty minutes or more, at which time the solutions were removed. Tested in this way, cocain solutions were but slightly if at all irritating, and within a few minutes their application was followed by distinct evidence of anesthesia; for example, to application of normally painful mechanical stimuli, the area showed a diminished sensibility. We, therefore, cannot accept Wiki's conclusions upon the evidence he has presented.

He states that isotonic solutions of the magnesium salts are not without anesthetic action, but stronger solutions give more intense effects. These results would speak against an infiltration anesthesia such as we know can be produced by distilled water or even isotonic sodium chloride solutions. But in view of our results which demonstrate so clearly the pronounced irritating action of hypertonic magnesium salt solutions applied to abraded skin areas and complete absence of anesthetic symptoms, we are inclined to interpret Wiki's observations as evidence of infiltration anesthesia or inhibition of the reflexes through sensory stimulation.

Though aware that such experiments conducted by application of the agents to human cutaneous abrasions have been criticized especially from the psychological standpoint, yet we would emphasize their importance when carefully conducted and controlled, for not only is it possible to conduct a large series of observations in a relatively short time, but the evidence secured, being direct, is final.

Since all of the inorganic salt solutions employed (which include the chlorides and sulphates of Na, K, NH_4, Mg, and the chlorides of Ca and Ba) have similar actions, the difference being, so far as has been observed, a quantitative one, the action would seem to be more of a physical or physical chemical than of a chemical or specific nature. With hypertonic solutions the differences ob-

served in action correspond to the difference in concentration. Also, for a given degree of action the molecular concentration is not the same for all substances, and though the evidence has not yet been completely analyzed, it seems to be in general agreement at least, with the views of Meltzer and his pupils,[1] that the toxicity of solutions of salts that naturally occur in the blood varies inversely with the amount in which they are thus present.

90 (907)

The blood in "shock."

By **C. C. GUTHRIE** and **F. V. GUTHRIE** (by invitation).

[*From the Physiological Laboratory, University of Pittsburgh.*]

It has been stated that in conditions of "shock" concentration of the morphological elements of the blood may take place through outward passage of liquid from the blood vessels.[2]

This point was investigated in a rather comprehensive experimental study of "shock" in etherized dogs. This condition was induced by rhythmical Faradic stimulation of the brachial plexuses and moderate hemorrhage. In general, death occurred within one or two hours. Small samples of the blood were withdrawn at regular intervals and defibrinated. The specific gravities and freezing points of the blood specimens were measured in eight experiments. The results are practically the same in all cases, and show that under the conditions of the experiments, physical alterations in the blood are not greater than may be accounted for by the loss of blood and certainly are not such as could affect the circulation sufficiently to account for the phenomena observed.

Relative to the total mass of blood, the amount withdrawn was, in round numbers, between 15 and 30 per cent., the average being 25 per cent.—estimated on the total blood being 1/15 of the body weight.

The average arterial blood-pressure at the time the first blood sample was taken was 180.5; at the time of taking the last sample

[1] *Jr. Pharm. and Exp. Ther.*, 1909, I, 1.

[2] Malcolm, *Lancet*, 1905, II, 573; 1907, I, 497.

47 mm. Hg pressure. The average specific gravity of the first sample was 1.059; and of the last 1.056. The average change in freezing point was + 0.016.

Viscosity measurements were made in a few experiments. In this respect also there was insufficient evidence for attributing the clinical condition of the animal to mechanical change in the blood.

91 (908)

Laking of blood by hypertonic solutions.

By **C. C. GUTHRIE** and **M. E. LEE** (by invitation).

[*From the Physiological Laboratory, University of Pittsburgh.*]

It is known that hypertonic solutions when added to blood may cause laking.[1] To determine if possible if this phenomenon could be due to a drying action on the scarlet blood discs, as this is known to so affect them that they lose hemoglobin to watery solutions even though such solutions are iso- or hyper-tonic to blood serum, experiments were performed to observe the action of hypertonic solutions of a number of relatively inert inorganic salts and other substances, including the chlorides of Na, K, Mg, Ca and Ba; the sulphates of Na, K, Mg; cane sugar and glycerine. For in drying through evaporation the salt content of the liquid surrounding the cells must become decidedly concentrated before all of the water has evaporated. The results of the observations may be summarized as follows:

1. Laking by hypertonic sodium chloride solutions or by hypertonic solutions of other inert salts is proportional to the concentration of the solution.

2. In hypertonic solutions of inert substances in equimolecular concentrations, laking is not the same in all. And consideration of the isotonic coefficients of such substances does not indicate that laking is altogether due to osmotic strength.

3. In equimolecular hypertonic concentrations, the chlorine salts are more powerful than the corresponding sulphates.

4. Non-electrolytic solutions, as cane sugar and glycerine, in hypertonic concentration produce laking, and it is proportional to the concentration of the solution.

[1] Bursy, Inaugural-Dissertation, Dorpat, 1863.

92 (909)

Laking of blood by freezing and thawing.

By C. C. GUTHRIE and M. E. LEE (by invitation).

[*From the Physiological Laboratory, University of Pittsburgh.*]

The mechanism of laking by freezing and thawing[1] is not understood. Since it is known that drying through evaporation will cause laking, it is possible that drying through crystallization of water[2] may account for laking by freezing. But again since it is known that hypertonic solutions may cause laking, and since there is evidence that the freezing point of serum is somewhat higher than that of the intracellular liquids and therefore in freezing a concentration of the serum solids occurs, it may be that such laking is fundamentally the same as laking by hypertonic solutions.

It was observed that blood could be repeatedly frozen and thawed as in freezing point measurements with slight or no laking. But when blood was exposed a single time to a temperature considerably below the freezing point for some time, on thawing strong laking occurred. To gain information on the question as to what degree of cooling is necessary to cause laking and also as to the relation of time of exposure to such temperature to the degree of laking, the experiments, the results of which are herein reported, were performed. The blood of various animals including ox, dog, cat, and fowl has been used. Thus far no marked differences in the behavior of the different bloods have been observed. Summarized, the results show:

1. That slight or no laking occurs when the temperature of the blood is sufficiently lowered for the formation of crystals of ice if the blood is maintained at this temperature for but a short time.

2. When maintained at a temperature between a point slightly lower than that of the freezing point and minus one degree centigrade for ten minutes or more, laking occurs after the tube has remained at room temperature for some time.

3. The degree of laking, within limits, is proportionate to the

[1] Rollett, *Sitzgsber. d. Wiener Acad.*, XLVI, S, 65, 1862.

[2] Cf. Müller-Thurgan, *Landw. Jahrb.*, XV, 534, 1886.

degree to which the temperature was lowered, and to the length of time during which the low temperature was maintained.

93 (910)

Laking of blood by drying.

By C. C. GUTHRIE and M. E. LEE (by invitation).

[*From the Physiological Laboratory, University of Pittsburgh.*]

Drying of blood may cause laking of the scarlet blood discs.[1] To obtain more information regarding the phenomenon, the expeiments reported in this communication were undertaken.

The phenomenon may be produced in a number of ways. Perhaps the simplest is to prepare an ordinary wet blood mount and to observe it with a microscope. Another method is to make an ordinary blood smear and allow it to dry quickly in the air and then place it film side down on a microscope slide and after focusing it under a microscope, place a drop of serum, salt solution or other liquid on the slide, so that the edge of the drop forms contact with the edge of the slip and will spread under it.

Under these conditions laking occurs and may be readily observed in the individual discs.

In the case of a wet mount, the process is slow and various stages may be seen. If drying be rapid the individual discs may lose their hemoglobin with slight or no change in size. But if it be more gradual, they may be seen to swell before laking. This is also the case with shrunken or crenated discs.

In the case of dry mounts, the hemoglobin is almost instantaneously dissolved on contact with the liquid under the slip. If any change in the size of the discs occurs, it has not been observed.

At present the manner in which laking is thus produced is not known. Drying is, of course, accompanied by concentration of salts or other substances present in solution or suspension both within the discs and in the serum. Now it is known that hypertonic solutions of such substances if caused to act on the blood may produce laking. Therefore, the question arises, Is laking

[1] *Am. Jr. Phy.*, 1903, VIII, 441.

through drying the same fundamentally as laking by hypertonic solutions? Brahmachari[1] suggests that hypertonic solutions of sodium chloride lake by uniting with some cellular constituent and thus alter its normal properties. If this is true, it would seem that such union is at least of a doubtful chemical character; for a number of inorganic salts as well as cane sugar and glycerine in hypertonic solution may cause laking.

It is conceivable that the abstraction of sufficient water from the discs in any manner as by evaporation or by hypertonic solutions or by freezing, may so alter the molecular arrangement of the essential structures that it is not possible for them subsequently to imbibe or otherwise take up water and regain their normal properties,—hence laking.

94 (911)

The schizogony in the life-cycle of Sarcocystis muris.

By RH. ERDMANN.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

The first period of the life cycle of *Sarcocystis muris* extends from the date of infection to the entrance of the unicellular parasite into the muscular tissue of the host (20 to 30 days).[2] In my former publications I could only describe from this period some large ameboid forms found in the walls of the intestine and in the lymph vessels of experimentally infected mice.[3]

My present investigation shows the appearance of small ameboid and schizogony forms six days after infection. These stages were discovered after feeding sarcosporidia to young mice nourished with milk from birth to the end of the experiments. These small schizogony forms (0.3 to 0.4 μ) consist of a tiny protoplasmic body with a caryosome-nucleus, and arise from smaller ameboid organisms which show typical schizogony.

[1] *Bio-Chem. Jr.*, 1909, IV, 59.

[2] Erdmann, "Die Entwicklung der *Sarcocystis muris* in der Muskulatur," *Sitzungsberichte der Gesellschaft Naturforschender Freunde*, 1910, p. 399.

[3] Ibidem, p. 382. Also cf. Erdmann, "Beiträge zur Morphologie und Entwickelungsgeschichte des Hammelsarkosporids in der Maus," *Centrb. für Bakt. und Parasitk.*, 1910, Bd. 53, Abt. I Orig., p. 515.

Although these mice did not contain any other protozoan parasites in the intestine, I hesitate to connect positively the small ameboid and schizogony forms with the newly introduced Sarcosporidian "spore" until further study actually demonstrates the transition.

A complete account of the work will appear in the *Arch. d. Zool. expér. et gén.*, T. 52, 1914.

95 (912)

The purine enzymes of the anthropoids and marsupials.

By H. GIDEON WELLS and GEORGE T. CALDWELL.

[*From the Otho S. A. Sprague Memorial Institute and the University of Chicago.*]

Previous studies have shown that the human organism contains no enzymes which will destroy uric acid *in vitro*, in which respect man differs from all other mammals hitherto investigated. This corresponds with the repeated observations, especially of Wiechowski, that man alone of all domestic mammals excretes uric acid rather than allantoin as the chief end product of purine metabolism. These facts have been especially emphasized of late by Andrew Hunter. One of us found that even the monkey has no demonstrable uricolytic enzymes in its tissues. Wiechowski made the interesting observation that the chimpanzee, like man, excretes only uric acid and little or no allantoin, while Hunter and Givens reported that monkeys resembled the other mammals in excreting chiefly allantoin, corresponding with our observations on the purine enzymes of the monkey. We have recently, through the kindness of Dr. W. T. Hornaday of the New York Zoölogical Society, come into possession of two fresh bodies of anthropoids—a male chimpanzee and a female orang-utan. Examination of their tissues shows that, like man, they do not possess the uricolytic enzyme, uricase, demonstrable *in vitro*. They also resemble adult man in having guanase but no demonstrable adenase. Hence it seems that the anthropoids stand with men in constituting, in respect to uricolytic power, an exception to all other known mammals; the monkeys resemble the other lower mammals in

possessing uricase, and hence in this property the anthropoids stand closer to man than to the monkeys, as they are also said to do in serological reactions. We have found a marsupial, the opossum, to have uricase, xanthine oxidase, guanase but no adenase. In respect to uric acid destruction our results agree perfectly with the urinary analyses of Hunter and others.

96 (913)

A quantitative application of the Abderhalden serum test.

By DONALD D. VAN SLYKE and MIRIAM VINOGRAD.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

The Abderhalden serum test can be greatly simplified, made quantitative, and the sensitiveness increased about thirty-fold compared with the dialysis test, by utilizing the nitrous acid method to detect proteolysis. The technique is the following: 2 c.c. of serum are digested with 0.1 gram of dried substrate (tissue prepared according to Abderhalden's directions and dried quickly at 0.5 mm.), or, as nearly as can be estimated 0.4 gram of undried substrate. After the digestion is complete, 3 c.c. of water are added. The solution is then centrifugated, and 2 c.c. used for amino nitrogen determination in the micro-apparatus, 0.5 c.c. of caprylic alcohol being used to avoid foaming, and the reaction being run four minutes. Control analyses are run under the same conditions with serum that has been digested with normal tissue, and with no tissue. The amino method will detect one fourth the concentration of α-NH_2 that is apparent by the ninhydrin reaction, and the serum is diluted only one eighth as much in the above procedure as in the dialysis test, so that the sensitiveness is increased about thirty-fold. In spite of this, our results have been absolutely negative with the Rous chicken sarcomas Nos. 1 and 2, even when serum tested was from the chicken furnishing the tissue substrate. The results do not, of course, bear on the validity of the test in human cases. We are about to test the method in human cancer and in pregnancy. The work on chicken tumors has been possible as a result of the cordial coöperation of Dr. Rous and Dr. Lange.

97 (914)

The mode of action of urease. II.

By DONALD D. VAN SLYKE, GOTTHARD ZACHARIAS and GLENN E. CULLEN.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

The action of the generated ammonium carbonate in retarding the action of urease is due to the alkalinity of the carbonate. When the solution is kept neutral by a proper phosphate mixture the products have no effect on the velocity of the reaction. Elimination of the effect of the products makes urease a particularly favorable enzyme with which to study the reaction between enzyme and substrate. The results indicate that the action consists of two successive reactions: combination of enzyme and substrate in definite proportions; and decomposition of the compound, the urea being thrown off as ammonium carbonate; each of the two reactions consuming a definite portion of the total time. Formulation of these relations leads to the equation $t = \frac{1}{c} \log \frac{a}{a - x} + \frac{x}{d}$, t representing the time required for the decomposition of x amount of the initial substrate amount, a; c is a constant representing the velocity of combination of enzyme and substrate, d representing the velocity of decomposition of the complex. The values of c and d can be determined independently, and one can thereby determine whether changes in conditions affect the combination reaction, or that of decomposition. Neutral salts retard the combination. Alkaline reaction hastens it, but retards the decomposition. Slightly acid reaction greatly retards the combination, affecting the other reaction but little. The independent variation of the two phases of the process of enzyme action explains some previously obscure facts in regard to the effect of alkalies, acids, and other substances on enzyme action.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Sixtieth meeting.

Carnegie Laboratory for Experimental Evolution, Cold Spring Harbor, June 6, 1914. President Lusk in the chair.

98 (915)

Extra sugar during ether and nitrous oxide narcosis in fully phlorhizinized dogs. Sources of error in existing methods for the study of gluconeogenesis.

By **W. D. Sansum** and **R. T. Woodyatt.**

[*From the Otho S. A. Sprague Memorial Institute Laboratories for Clinical Research, Rush Medical College, Chicago.*]

Dogs were prepared by injecting subcutaneously 1 gram of phlorhizin suspended in olive oil once every twelve hours until the G : N ratio was constant, then every 24 hours.

It has been observed by Lusk[1] that fasting and phlorhizin alone do not remove all glycogen from the body. This was confirmed for dogs prepared in the manner described. Such animals were narcotized with ether, nitrous oxide and other narcotics. Narcosis with ether and nitrous oxide was always followed by a large increase of the glycosuria with a rise in the G : N ratio. When ether and chloroform were used, there was as a rule, a fall in the output of nitrogen and acetone bodies and a fall in sugar following the initial rise, but with nitrous oxide the rise in sugar was unaccompanied by such changes. In one of the ether experiments also the fall in nitrogen was very slight and there was no lessening of the glycosuria following the initial increase.

An animal prepared in the usual way was given a cold bath for 20 minutes followed by 6 hours of moderate shivering. After this 1 mg. of epinephrine was given intravenously once every 6 hours.

[1] *Ergeb. der Physiol.*, XIII, p. 361 (1912).

At first there was a marked increase in the glycosuria, but ultimately the G : N ratio resumed the normal level and was little affected by epinephrine. Nitrous oxide narcosis was then induced as before. The extra sugar for 24 hours was now 1.8 g. as compared with a minimum of 7 g. in two previous experiments.

It is concluded that the "extra" sugar which is eliminated when phlorhizinized dogs are narcotized with ether and nitrous oxide has its origin in glycogen; that the increase in glycosuria incidental to narcosis is a phenomenon which can occur independently of any fall in nitrogen or decrease of the acetone body output. The lessened nitrogen and acetone excretions and the late fall in sugar following ether or chloroform administration suggest renal injury with general retention; or, possibly, they are due in part to suppression of the total metabolism. In any case they depend upon some action of the narcotic separate in kind or in degree from that which is responsible for the appearance of extra sugar. It is thought that asphyxia alone will produce results comparable to those seen with nitrous oxide.

The question arises as to whether acetaldehyde, pyruvic acid and some of the other substances which have been reported to increase the glycosuria in diabetic dogs prepared by the methods used in these experiments, may not do so by causing a mobilization of glycogen in a manner similar to that which follows the administration of the better known narcotics (general asphyxia or impairment of tissue respiration?). In the study of gluconeogenesis in diabetic dogs—especially when the substances administered are chemically active or possessed of pharmacodynamic properties, which is the case with short chain aldehydes, ketones, keto acids, etc.—the appearance of extra sugar in the urine affords no proof that the substance itself has been converted into glucose or that any *new* sugar has been formed from any other source (such as fat), unless suitable methods have been employed to eliminate the glycogen. If during the fore period preceding the experiment period a dose of epinephrine were given without causing the appearance of extra sugar, this would make it reasonably certain that a subsequent rise in sugar was not referable to an hydrolysis of glycogen. This is suggested as a convenient method of checking the effectiveness of the preliminary

regime. The criticism which may be made of experiments with short chain aldehydes, ketones, ketoacids, etc., in dogs prepared by fasting and phlorhizin alone are not applicable to the results obtained with animals which have been subjected to cold until all glycogen has been exhausted; nor is it necessarily implied that alanine, aspartic acid, lactates, propionates, and many other bland substances which have been studied might not yield entirely satisfactory results even in the presence of a residue of glycogen.

99 (916)

On the difference in the response of animals of different ages to a constant quantity of uranium nitrate.[1]

By WM. DEB. MACNIDER.

[*From the Laboratory of Pharmacology, University of North Carolina.*]

The following report is based upon the difference in the response of forty-eight animals of different ages to a constant quantity of uranium nitrate.

Dogs have been employed in all of the experiments. The animals have varied in age from puppies of four months old, to animals of extreme old age, one of the animals having reached the age of twenty years.

All of the animals have received uranium nitrate in the dose of 6.7 mgs. per kilogram on two successive days. The uranium was given subcutaneously.

The animals were fed on raw meat and bread.

In a recent publication[2] it has been shown that when a constant quantity of uranium nitrate is given to young and full grown animals, that the age of the animal influences the total output of urine and also the composition of the urine. The total output of urine in a twenty-four hour period was greater in the adult animals. The percentage of glucose in the urine (Benedict determination) was greater in the adult animals than in the puppies.

In the urine of the puppies acetone was either absent or present

[1] Aided by a grant from the fund for Scientific Research of the American Medical Association.

[2] MacNider, *Jour. of Pharm. and Exper. Ther.*, IV, 6, 1913.

in very small amount. The urine of the adult animals invariably contained acetone.

The present series of forty-eight animals shows the same differences in the total output of urine and in the composition of the urine as has been above referred to.

In addition, these animals of different ages show certain other characteristics which are apparently dependent upon the age of the animal.

1. Ten of the animals were puppies varying in age from four to eight and a half months. Only one of these animals developed a glycosuria within the first twenty-four hours following the initial injection of 6.7 mgs. of uranium. The percentage of glucose in the urine of this animal was 0.103 per cent.

2. In none of the puppies was acetone present in the urine following the first injection of uranium.

3. Following the second injection of uranium all of the puppies developed a glycosuria. The percentage of glucose varied from 0.35–1.1 per cent.

4. Following the second injection of uranium only two of the puppies showed the presence of acetone in the urine. The amount of acetone was exceedingly small.

The full-grown animals and old animals have shown the following differences in their response to the same quantity of uranium per kilogram that was received by the puppies.

1. With three exceptions, the remaining thirty-eight adult animals all developed a glycosuria within twenty-four hours following the first injection of 6.7 mgs. of uranium. The percentage of glucose varied from 0.18–1.61 per cent. The three highest percentages of glucose—1.61, 1.59 and 1.24 per cent were obtained in old animals.

2. Following the second injection of uranium all of the full grown animals became glycosuric. The percentage of glucose varied from a minimum of 1.47 per cent to a maximum of 2.86 per cent.

3. Acetone was present in the urine of all of the full grown animals following the first injection of uranium excepting the three animals that failed to develop a glycosuria following the first uranium injection.

That the above differences in the response of puppies and adult animals to a constant quantity of uranium are not dependent upon the relative weight of the different animals and therefore associated with the total amount of uranium received by the animal but are associated with the difference in the age of the animals is clearly shown by the following experiments:

Experiment 18.—Puppy, aged 7 months. Weight 9.35 kilos. The animal received 6.7 mg. of uranium nitrate per kilogram on two successive days. The urine following the first injection contained neither glucose nor acetone. Following the second injection of uranium the urine contained 0.35 per cent. glucose and a trace of acetone.

Experiment 38.—Full-grown animal, old, weight 7 kilos. Following the first injection of 6.7 mg. of uranium nitrate per kilogram the urine contained acetone and the animal developed a glycosuria. Glucose was present in 1.21 per cent. Following the second injection of uranium the amount of acetone was apparently greatly increased. Glucose in the urine had increased to 2.84 per cent.

The experiments were terminated by either shooting, or killing the animals with chloroform or ether.

In the account which is to follow of the fatty changes which are induced in the liver and kidney of the animals of different ages, none of the animals which were subjected to the effect of an anesthetic will be included. As has been previously shown the anesthetic very greatly increases these changes.[1]

Frozen sections were made at once and stained by Herxheimer's Scharloch R method for fat. The sections were counter stained by Mayer's Haemalum. It is important for both of these stains to be fresh.

The frozen sections have shown that the amount of fat in the liver and in the kidney of puppies which have been given uranium nitrate is very much less than in the adult animals. The fat in the liver is found as dust-like particles which serve to outline the bile capillaries while the epithelium of the interlobular bile ducts contains larger quantities of fat in the form of coarse granules. The cytoplasm of the liver cells contains numerous small fat droplets, but the principal localization of the fat is either within the

[1] MacNider, *Jour. Med. Research*, XXVIII, III, 1913.

bile capillaries or in that zone of the cell which immediately surrounds the capillaries.

In the kidneys of these animals the fat first appears in the loops of Henle. The fat is most pronounced in the ascending limb of Henle's loop.

In puppies and young dogs the fat is in small amount and appears in the form of minute granules in the epithelium lining these tubules. In adult animals and especially in old animals the fat is very greatly increased in amount and is seen in the form of large granules which may coalesce to form masses which serve to outline the course of the tubule.

There is apparently an association between the amount of fat found in the liver and kidney of animals of different ages with the amount of glucose present in the urine. The puppies and young animals which show a low percentage of glucose in the urine show a small amount of fat in the liver and kidney.

The full-grown animals and old animals which have shown an earlier appearance of glucose in the urine and a percentage of glucose which has been much higher than has been found in the puppies also show fatty changes in the liver and kidney of much greater severity.

100 (917)

Variations in resistance of red blood cells in sheep.

By R. OTTENBERG and J. G. HOPKINS.

[*From the Department of Bacteriology, College of Physicians and Surgeons, N. Y.*]

In the course of complement fixation work we have noticed that the specimens of blood cells obtained from different sheep under exactly similar conditions occasionally show marked differences in their susceptibility to laking by specific lytic serum. When this was first noticed we were using two sheep as a source of blood, and as the sheep whose cells were more highly resistant was one which had been bled repeatedly and profusely it was natural to attribute the increased resistance of the cells to this.

We determined to investigate this question and also to find out whether the increased resistance was a specific resistance to

laking by amboceptor and complement or was a general resistance such as would be shown by an increased resistance to laking by hypotonic salt solution, and also whether the sera of different sheep show similar differences in their tonicity.

Three sheep were used:—

T—an apparently normal young male sheep, newly obtained from the dealer.

F—a sheep which had been kept in the laboratory for six months and had been bled repeatedly and on occasions profusely.

P—a sheep which had been confined for two and one half years, had been bled occasionally and had received immunizing injections of typhoid bacilli for a period of about eighteen months.

F was slightly and *P* decidedly anemic. The blood of all three was obtained on the same day and the cells were washed with great care to handle the blood of all three animals in precisely similar ways. All three were made up into 5 per cent suspension and tested with diminishing quantities of hemolytic amboceptor (rabbit serum) and a fixed dose of 10 per cent complement (guinea pig) with the following result:

TABLE I.

VARIATIONS IN RESISTANCE TO LYTIC SERUM.

Each tube contains 0.5 c.c. 5 per cent. washed red cells, 0.1 c.c. of 10 per cent. guinea-pig complement and the amount of immune serum indicated below. Incubation one-half hour.

(1/600) Amboceptor.	.1	.15	.2	2.5	3.0
Cells of sheep *P*.....	+++	++++	++++	++++	++++
Cells of sheep *F*.....	++	+++	++++	++++	++++
Cells of sheep *T*.....	+	++	++++	++++	++++

(++++ = complete hemolysis.)

It is seen that the new sheep, *T*, was the most resistant to laking, the frequently bled sheep, *F*, almost as resistant, and the typhoid injected sheep, *P*, least resistant.

At the same time the washed cells were tested for their susceptibility to laking by anisotonic salt solutions, by adding .2 c.c. of 30 per cent suspension of cells of each sheep to 5 c.c. of salt solution whose strength ranged from .3 per cent to 9 per cent with intervals of .025 per cent.

The new sheep (*T*) was then bled 800 c.c. and the blood of all

three animals again tested on the first, second and sixth day after this bleeding.

For the sake of brevity the complete data obtained with the salt solutions are not given but only the point of beginning hemolysis and of complete hemolysis. They are as follows:

TABLE II.

Date.	Hemolysis begins.	Hemolysis complete.
	Sheep T.	
April 22	.600	.400
April 23	.575	.400
April 24	(.525)	.400
April 28	.575	.400
	Sheep F.	
April 22	.600	.450
April 23	.575	.450
April 24	(.525)	.450
April 28	.575	.450
	Sheep P.	
April 22	.700	.525
April 23	.675	.500
April 24	( )	
April 28	.650	.525

It is seen that hemorrhage had practically no effect on sheep *T* (the variations are within the range of experimental error). The resistance of each animal was about the same on each day.

The cells on each day were also again tested with amboceptor and complement and without giving charts of the results it may be stated that the differences between the three sheep were substantially the same as they were before the bleeding of sheep *T* on each of the four days.

It is interesting to note that the resistance to immune serum on each day ran parallel to the resistance to hypotonic salt solution, the cells of sheep *P* always being most easily laked, those of sheep *F* next and those of sheep *T* least easily laked.

We also attempted to determine whether the differences in susceptibility to laking by immune serum were due to differences in ability of the different sheep's cells to absorb amboceptor. This was found not to be the case: at least the cells of all three sheep were able to absorb a great excess (at least 30 units) of

amboceptor, and likewise the titrations of the different cells against immune serum gave the same relative results whether the amount of immune serum was kept constant and the amount of complement varied, or the amount of complement was kept constant and the amount of immune serum varied.

The serum of the three animals was also tested for its tonicity by diluting with graded amounts of distilled water and adding to 2 c.c. of each dilution .1 c.c. of 30 per cent suspension of freshly washed cells from sheep *F*. The serum of sheep *T* did show a very slight lowering of its tonicity following the hemorrhage. The sera obtained on three different days were tested simultaneously.

TABLE III.

HEMOLYSIS OF RED CELLS OF SHEEP *F* IN SERUM OF *T* DILUTED WITH DISTILLED WATER.

Per Cent. of Serum.	50	52.5	55	57.5	60
Serum of *T*, April 22	++	+	+	−	−
Serum of *T*, April 23	+++	++	+	+	−
Serum of *T*, April 24	+++	++	+	+	−

(++++ = complete hemolysis.)

The tonicities of the sera of the three animals were very nearly the same and had no relation to the rather wide variations in the resistance of their red cells.

Smith and Brown working with horses' blood found marked variations from the average resistance to salt solutions of low tonicity. About 10 per cent of horses have red cells very sensitive to hypotonic solutions. The sera of these horses were rather constant in tonicity. Cornwall[1] found that the apparent tonicity of sheep serum has no relation to the mean lytic point of the red blood corpuscles of the individual and is largely due to lipoids. He also found marked variations in individual animals.

Experiments of different workers on the effect of hemorrhage on the resistance of blood cells have given varying results. Smith and Brown[2] working with horses found a slight decrease in the resistance to hypotonic salt solution only after many large hemorrhages and only in some individuals. Itami and Pratt[3] also working with rabbits, found a slight increase in resistance.

[1] Cornwall, *Jour. of Hygiene*, Oct., 1912, Vol. 12, p. 245.

[2] Smith and Brown, *Jour. Med. Res.*, 1906, Vol. 15, p. 415.

[3] Itami and Pratt, *Biochem. Zeit.*, 1909, Vol. 18, p. 302.

The work of a great many different authors has shown that when anemia is produced by hemolytic poisons the resistance of the blood cells is increased, and it is generally accepted that in the pernicious and hemolytic types of human anemia the resistance of the red cells is increased whereas in secondary anemias it is diminished.

From our experiments we conclude that the red cells of individual sheep show marked variations to laking either by immune serum or by hypotonic salt solution and that resistances by laking by these two agencies are always parallel to each other. These differences are not due to acute hemorrhage. Whether they are due to differences in race or to differences in hygienic conditions (prolonged confinement, immunization with typhoid bacilli) we cannot yet state. During a short period of observation (about a month) in the case of two of the sheep, the cells of each animal were practically constant. There is a slight diminution in the tonicity of the blood serum immediately after an acute hemorrhage: this is possibly due to the fact that the body can more rapidly obtain water than it can salt and other serum constituents. The apparent tonicity of the serum has no relation to the tonicity of the red cells of the individual.

101 (918)

The influence of decerebration on the convulsant action of caffein in frogs.

By T. S. GITHENS.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

It is well-known that destruction of the brain causes increase of reflex action in frogs, especially when they are kept in the cold, and last year I reported that the effect of morphin, which causes in frogs tetanus indistinguishable from that of strychnin, was very markedly increased by decerebration, the effective dose in such frogs being about one tenth of that in normal frogs.

I wish to report now on the result of a study of the effect of decerebration upon the convulsant action of caffein. Caffein salts

which are strongly acid cause a peculiar muscular stiffness which masks the central action. The alkaloid itself causes also a certain muscular stiffness but it is much less marked and is only troublesome with large doses. The alkaloid was therefore used in these experiments. As was the case with other convulsant drugs we found the action of caffein much greater in the cold. If the frogs are kept cold tetanus can be obtained with doses which are too low to markedly affect the muscles.

In contrast to morphin we found that both in the cold (2–5° C.) and in the warm (12–18° C.) there was no difference between normal and decerebrate frogs in their response to the convulsant action of caffein. In both tetanus was obtained in the cold in all frogs with doses of 0.2 mg. p. gm. and in most frogs with 0.1 mg. p. gm. At room temperature tetanus was constantly obtained in normal decerebrate frogs with doses of 0.6 mg. p. gm. but never in either with 0.3 mg. p. gm.

The experiments show that there is a difference of some sort between the actions of caffein and morphin on the central nervous system although the convulsions are identical in appearance.

102 (919)

On the production of hyaline casts by certain ions.

By F. L. GATES and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

At the February meeting of this Society one of us (G.) reported that after an intravenous or intramuscular injection of a sublethal dose of a solution of magnesium sulphate in dogs hyaline casts invariably appear in the urine. As a result of this observation a series of experiments was made to answer the question as to which of the ions of the injected salt is the cause of the appearance of the hyaline casts—the kation magnesium, or the anion SO_4, the sulphate radical. We have tested in the first place several magnesium salts as well as several sulphates. This led up to further experimentation with some salts which contain neither magnesium nor the sulphate radical. Briefly stated, our results are in general

as follows: The injection of any compounds which have either magnesium or the sulphate radical as a component caused the appearance of hyaline casts in the urine. The injection of salts which had in their composition neither Mg nor SO_4, produced, however, no such effect. Of the magnesium salts, besides the sulphate, the chlorid, nitrate and the acetate were studied also. They all caused the appearance of hyaline casts in abundance. The acetate was perhaps less effective. Of the sulphates, besides the magnesium, also the salts of sodium, ammonium, and potassium were studied. All gave hyaline casts; the action of potassium, however, was less transparent. On the other hand the chlorids of sodium and of ammonium and the nitrate and the acetate of sodium produced no hyaline casts. The effects of potassium salts were apparently complicated by the profound action of these salts upon the heart and probably also by some direct action upon the kidneys. Of course, we could not attempt to give here any further details, nor enter upon a theoretical discussion of the possible significance of the reported facts. We wish only to add the statement that an analysis of the experiments seems to show that there is some definite relation between the diuretic action of the salts under discussion and their specific capacity for producing hyaline casts.

103 (920)

The specific dynamic action of levulose, glycocoll and alanin in phlorhizin glycosuria.

By GRAHAM LUSK.

[*From the Physiological Laboratory of the Cornell University Medical College, New York City.*]

Ingestion of levulose by a dog which has been phlorhizinized does not increase the metabolism; the respiratory quotient is not changed and levulose is converted into dextrose, for this alone appears in the urine and in increased quantity. Ingestion of glycocoll or alanin largely increases the metabolism in glycosuria, although they are not oxidized and are converted into glucose and urea. The conclusion is drawn that the preliminary cleavage products of carbohydrate break-down are not stimulants of metabolism,

but that the increased heat production following carbohydrate ingestion is due to the mass action of sugar molecules. On the contrary, amino-acids or the oxy- or keto-acids produced from them act as stimuli upon the cells thereby increasing the heat production.

104 (921)

The production of duodenal lesions and ulcers in dogs by injections of epinephrin and of gastric lesions and ulcers in rabbits by extirpation of the adrenals.

By G. A. FRIEDMAN, M.D.

[*From the Department of Clinical Pathology, Columbia University.*]

In several papers I have pointed out the frequency of polycythemia or polyglobulia in patients suffering with non-bleeding duodenal ulcer. I have also called attention to the presence of eosinpenia in such patients. In gastric ulcer occurs just the reverse anemia and relative eosinophilia.

Of eighteen operatively demonstrated cases of duodenal ulcer, in fifteen polyglobulia was found, while of twelve cases of gastric ulcer, polycythemia was noted only in one.

For details I refer to my article, which will soon appear in the *Journal of the Am. Med. Sc.*

The question arose, what has duodenal ulcer to do with polyglobulia. As several investigators who injected adrenalin in dogs and in men, have found an increase in erythrocytes from 30–70 and 100 per cent (this experimental polyglobulia lasting sometimes about thirty hours) and also a reduction in the number of eosinophiles, the idea has gained access to us, that the initial lesion of duodenal ulcer may be caused by hyperfunction of the adrenals.

As in none of our patients with duodenal ulcer high blood pressure was noted, the assumption was, that in such patients a slight excess of epinephrin may circulate in the blood in quantities not sufficient to cause hypertention, but enough to exert its hormone influence upon the duodenal mucosa.

I have succeeded in producing duodenal lesions and ulcers in eight dogs by subcutaneous and intravenous injections of adrenalin hydrochloride in a period of about one to two weeks daily (with

one or two intermissions). Single doses did not exceed 3 mg. subcutaneously and 2 ½ mg. intravenously.

MACROSCOPICAL CHANGES FOUND IN OTHER ORGANS.

Stomach: ecchymoses in three dogs. Small intestines: marked congestion and hemorrhagic patches in two. Large intestines: slight hyperaemia in one. The appendix was atrophic and constricted in one. Lungs: Congestion of lower left lobe in one. Head of pancreas was found to be congested in two. Spleen was firm to the touch in one. Thyroidea, adrenals and thymus did not appear to be enlarged in any of the dogs.

The findings of duodenal lesions in each and in every dog could not be explained by mere coincidence.

In two dogs, used as controls, who were injected for about one week with thyroid extract, no lesions were found in the duodenum, but only in the stomach: erosion and ulcerations.

In one dog from whom the right lobe of the thyroid was extirpated and killed seven days later, two distinct soft ulcers were found at the first portion of the duodenum and no lesions were seen in the stomach.

I have also succeeded in producing gastric erosions and ulcers in rabbits by extirpation of one or both suprarenals. Autopsy was performed not before the seventh day after removal of the glands and not later than on the thirteenth except in two, who died.

FINDINGS IN FIVE RABBITS AFTER EXTIRPATION OF LEFT ADRENAL.

Erosions and ulcers at the pylorus in three, erosion at the pylorus and ulcer in first portion of duodenum in one, and no lesions in one. In the case of double ulcer the right adrenal was found to be nearly twice as large as normally; except new glandular tissue appeared at the site of the extirpated adrenal. In the case with negative findings fragments of new tissue were seen at the site of the extirpated gland and the right adrenal was found to be somewhat larger.

FINDINGS AFTER EXTIRPATION OF RIGHT ADRENAL.

Erosions at the lesser curvature, proximal to the cardia in three rabbits, a round ulcer at the posterior wall of the stomach, near

to cardia in one. The most pronounced ulcers, reaching down to the serosa, were obtained at the fundus of the tenth rabbit after extirpation of 2/3 of the right adrenal and five days later of the left.

In fourteen normal rabbits no lesions in the stomach or in the duodenum were seen.

Extirpation of left adrenal and right lobe of thyroid in one rabbit and right adrenal and left lobe of thyroid in another (in one sitting). Stomach and duodenum normal.

It is said that gastric lesions may be present in rabbits from infection. The latter two were the only ones who became infected and yet no lesions in the stomach were seen.

A full report of our work will be published in the near future.

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105 (922)

Mosaics and gynandromorphs in Drosophila.

By T. H. MORGAN.

[*From the Zoological Laboratory, Columbia University.*]

Quite a number of gynandromorphs and mosaics have appeared in our experiments with the mutant stocks of *Drosophila*. In some of these the male side or part is maternal, and in others paternal, and in one case both paternal and maternal. For the first type Boveri's hypothesis of partial fertilization will cover the result, for the second my own hypothesis of polysperm will explain the facts. In the third case the result must be due to mitotic dislocation at some early cleavage stage.

In order to test these three hypotheses, I crossed a female homozygous for the sex-linked characters, yellow body color and

white eyes, to a male with the normal sex-linked allelomorphs, viz., gray body color and red eyes, but in addition the male carried a recessive non-sex-linked character, viz., ebony body color.

One gynandromorph appeared among many thousand offspring. It was male on one side (partially) and female on the other. Both sides had red eyes and gray (or dark gray) body color. An analysis of this case shows that the male side must have contained the sex chromosome of its father and a non-ebony autosome from the mother. In other words, the gynandromorph on the male side is like the father except that it carries in addition one of the autosomal characters of its mother. The result means that at some early division a sex chromosome failed to pass to one pole and became lost.

Since this explanation will cover also the first two types, and since neither the hypothesis for the first nor that for the second type will explain all three types the third hypothesis is to be preferred. It leads to the conclusion that *gynandromorphs and mosaics may arise through a mitotic dislocation of the sex chromosomes.*

106 (923)

The applicability of Hermann's theory of alteration.

By R. BEUTNER.

[*From the Rockefeller Institute for Medical Research.*]

1. Herrmann's theory of alteration, which is accepted to-day by many physiologists, assumes that the junction of dead and of living tissue is the seat of an electromotive force which acts in such a direction that the dead tissue is negative while the living tissue is positive. This theory was advanced by Herrmann against DuBois Reymond who tried to explain the currents produced by muscles and nerves by means of complicated structural assumptions. Herrmann's theory has the advantage of expressing in an extremely simple form a large number of physiological observations. It has met general recognition partly also because all the opposing theories were of a very unsatisfactory character. The arguments however which Herrmann advances to support his views are not so conclusive as to fully justify his views. Especially his observation that a

definite time elapses between the cutting of the muscle and the appearance of the current of injury must not necessarily be due to a chemical alteration resulting from the injury. This time is so exceedingly short (5/1000 of a second) that a mechanical explanation rather suggests itself than a biochemical.

2. The conception that a difference of chemical processes in the dead and in the living tissue produces currents is also contradictory to physical laws. This was already pointed out by DuBois Reymond and his arguments seem still valid to-day. According to well-established physical principles an E.M.F. may be produced as a result of a chemical alteration which is brought by a current passing through the system, not however through a local chemical reaction which liberates ions.

3. Moreover certain experimental facts are in direct contradiction to Herrmann's theory; if a muscle is brought in contact at one end with distilled water and at the other end with a physiological NaCl solution it is found that the part in contact with the water is positive. This means that the injured part of the tissue is positive in this case since the distilled water has a destructive influence in direct contradiction to the alteration theory. Biedermann[1] who first observed this phenomenon has tried to bring it in harmony with the alteration theory. He argues that the death of the tissue produced by means of distilled water is of a peculiar nature because the conduction of irritation still persists under certain circumstances. This argument however certainly also contains hypothetical factors.

4. Mac Donald[2] has described interesting experiments which show that the magnitude of the current of injury has no relation to the state of life or death of the tissue. The current of injury of a sciatic nerve of the cat is measured immediately after excision and some time later; it is then generally found that the magnitude of the current decreases, which might be explained as being due to the death of the nerve. If however the nerve is immersed for a short time in a dilute salt solution the magnitude of the current of injury increases up to or above the original value. It hardly need be said that this treatment does not restore to the nerve any

[1] *Sitz. d. Wiener Akadem.*, 81, Abt. 3, 74 (1880).

[2] *Proc. Roy. Soc.*, 67, 310 (1900).

of its vital properties. This shows that the alteration theory can by no means claim a general applicability.

5. The alteration theory certainly does not give any point of view concerning the physical nature of the currents produced by tissues. Among the more recent attempts to solve this problem the so-called membrane theory is especially prominent. This theory was put forward by Ostwald in 1895 on the assumption that a semipermeable membrane is more permeable for cations than for anions. It is however not thoroughly justified *from a physical standpoint* either and Tammann and Walden who tried to prove it by means of analytical methods in Ostwald's laboratory have got contradictory results. Apparently therefore an experimental study of the E.M.F.'s produced by tissues and their artificial imitation, is a more promising method for the solution of the problem in question.

6. One of the most characteristic properties of the E.M.F. produced by tissues is the positivizing effect of water and of dilute salt-solutions as shown by the experiments of Biedermann and of Mac Donald described above. Dr. Loeb and the author have studied this phenomenon in a more quantitative way, namely with uninjured plants.

An artificial imitation of this property has been possible. A systematic study of cell arrangments composed of aqueous and water immiscible electrolytes (undertaken by the author) has shown that all water immiscible acid substances—used as ventral conductor show the same phenomena as living tissue, especially solutions of fatty acids in substituted phenols (as found recently by Dr. Loeb and the author).

The presence of a water immiscible acid (most likely a fatty acid) in the skin or membrane surrounding the tissue is therefore essential for this positivizing effect, not a selective permeability to cations as one would expect from Ostwald's theory. (Also the negativizing effect of K salts which was observed by Biedermann on muscles could be imitated by means of pure water immiscible substances.)

7. These experiments make it possible to investigate the physical nature of the single potential differences which compose the E.M.F.'s produced by tissues. For the cell arrangements of aque-

ous and water immiscible substances which imitate the tissue so far as the production of currents is concerned can be analyzed in all details much more easily than the tissue itself. The work along this line is not yet entirely finished, the results obtained so far show that biological potential differences are located at the junction of water immiscible fatty membranes and aqueous solution and that their magnitude is determined by peculiar phenomena of distribution. These phenomena of distribution can be fully accounted for by well-established physiochemical laws, but they are of rather complicated nature.[1]

The biological potential differences are not determined by ionic mobility as has been frequently assumed.

The methods used in this work are essentially physiochemical. The results obtained so far may appear insignificant to the physiologist as most of the more important electrophysiological observations especially those connected with irritation (action currents) remain unexplained. However the explanations for all these phenomena which the alteration theory can put forward are very hypothetical. It therefore seems justified at the present time to try a thorough explanation on the basis of well known physical laws of the very simplest electric phenomena observed in tissues.

107 (924)

The synthesis and rate of elimination of hippuric acid after benzoate ingestion in man.

By HOWARD B. LEWIS. (By invitation.)

[*From the Department of Physiological Chemistry, University of Pennsylvania, Philadelphia, Pa.*]

Ten grams of sodium benzoate were administered to a healthy man on a diet of milk, butter, and cane sugar, *i. e.*, glycocoll-free. The urine was collected at two hour intervals, and the relation between the elimination of hippuric acid and urea studied. As compared with the corresponding control periods on the same

[1] The details of this work are published in the *Journal of the American Chemical Society*, XXXV, 344 (1913), *Transactions of the American Electrochemical Society*, XXIII, 401 (1913), *Zeitschrift f. Electrochemie*, XIX, 319 (1913).

diet, there was observed a diminution of the urea-nitrogen eliminated during the first six hours after the benzoate ingestion, a diminution corresponding to the nitrogen eliminated as hippuric acid-nitrogen.

Periods.	Total Nitrogen.	Urea + NH_3 Nitrogen.	Undetermined Nitrogen.	Hippuric Acid Nitrogen.
I–III, control.....	3.521	3.074 (87.3%)	0.447	——
VIII–X, benzoate.....	3.760	2.436 (64.8%)	1.324 (0.481)	0.843

If the hippuric acid-nitrogen be subtracted from the undetermined N (shown in parentheses), the undetermined N is comparable with that of the control periods. This indicates that in man as in rabbits and pigs, the glycocoll available for synthesis into hippuric acid may be derived at the expense of substances whose N normally appears in the urine as urea-nitrogen. At the end of six hours the greater part of the hippuric acid had been eliminated and the urea elimination had become normal again.

No free benzoic acid nor glycuronates could be detected in the urine, indicating a complete conversion to hippuric acid and a very rapid elimination. In order to ascertain whether the rapidity of elimination was influenced by the liquid diet of the preceding experiment, the work was repeated on the same subject on a mixed diet, and on a purine-free diet. In both experiments, the greater part (85–95 per cent) of the hippuric acid was eliminated within six hours. Another subject received six grams of sodium benzoate and eliminated the greater part of the hippuric acid within six hours. An amount of sodium hippurate equivalent to the benzoate fed was administered, and the elimination of the hippuric acid studied. The rate of elimination was practically identical with that of the benzoate experiments. This indicates the great rapidity with which the human organism can detoxicate and eliminate a toxic substance such as sodium benzoate.

108 (925)

The production of accessory appendages and other abnormalities in amphibian larvæ through the action of centrifugal force.

By ARTHUR M. BANTA and ROSS AIKEN GORTNER.

[*From the Station for Experimental Evolution, The Carnegie Institution of Washington.*]

A year ago after centrifuging some eggs of *Rana sylvatica* it was noted that all of the survivors in one lot had accessory tail-like appendages. This seemed to be a sufficiently striking modification to merit some effort to learn just what was responsible for their occurrence. After many trials similar modifications were produced again this season. Eggs were treated in various stages from unsegmented eggs to the gastrula, and the different lots were given three different treatments.

In this note the earlier stages will not be considered. In a stage at which the blastopore had just become evident, or soon afterward became evident, the eggs which were subjected to a centrifugal force equivalent to 1,700 times gravity for two minutes were mostly killed. Accessory tail-like appendages developed in the survivors. 1,350 times gravity killed very few and accessory tail-like appendages developed in all of the survivors. 200 times gravity for ten minutes produced all normals in cases where the blastopore was not yet evident at the time of treatment. Where the blastopore was just evident all produced the accessory appendages. All survivors of similar treatments in the advanced gastrula and later stages were normal and accessory appendages were not produced in earlier stages than the blastula.

The accessory appendages, usually one to each animal although in some instances as many as four have been noted, occurred on different parts of the body. By far the commonest location was the mid-ventral region although they were in all positions from the anal region to the under side of the head. In many cases the position was more or less lateral, or even dorso-lateral, the appendage usually extending ventrally however. The appendages in the positions described were all distinctly tail-like having the characteristic myomeres seen in the normal tail and having a fin-like

keel on one or both margins. In some cases, nearly always in other lots of eggs than those which developed into larvæ having definite tail-like accessory appendages, the accessory structure was distinctly dorsal or cephalic in position. In some of the cases the structure was merely a broad-based, blunt, fleshy protuberance; in others it was wholly epidermal,—a more or less slender, sac-like epidermal projection. In still others the protuberance was longer, 1/8 to 1/10 the length of the larvæ, and extended distinctly forward from the frontal, dorso-frontal, or ventro-frontal region of the head. In some of these cases the myomere structure was evident although no fin-like margins occurred and the structures were approximately round in cross section.

In certain cases in which early gastrula stages of *Ambystoma punctatum* had been centrifuged, a number of the larvæ lacked the anterior region of the head including the eyes and, in fact, most of the head anterior to the gill region. A single lot of eight larvæ contained five possessing this abnormality.

Interpretations of these structures will be suggested in a larger paper.

109 (926)

Further observations on the toxicity of tin.

By WILLIAM SALANT and J. B. RIEGER.

[*From the Pharmacological Laboratory of the Bureau of Chemistry, Washington, D. C.*]

In a preliminary communication[1] from this laboratory, the results of experiments with the double salt of tin tartrate were announced. Experiments performed since with tin tartrate have shown that like the double salts, it caused marked injury to the kidneys. Large amounts of albumin were present in the urine of rabbits when administered subcutaneously and intravenously but larger doses of the normal salt were required to produce this effect.

The amount of tin as the double salt necessary to produce a very marked albuminuria was 20 to 30 per cent less than in the form of tin tartrate. Observations on the action of stannous and

[1] Salant and Smith, *J. Pharm. and Exp. Ther.*, 1914, Vol. 5, p. 517.

stannic salts failed to show any noteworthy difference. The effect of concentration indicated marked differences both when injected intravenously or subcutaneously. Thus 20 milligrams of tin per kilo, injected intravenously, produced a moderate albuminuria when the amount of tin per c.c. was 1.5 milligrams; much greater amounts of albumin and large amounts of sugar when each c.c. contain 8 milligrams of tin; acute death when each c.c. contained 20 milligrams of tin.

110 (927)

The influence of tartrates, citrates and oxalates on the isolated heart.

By WILLIAM SALANT and SELIG HECHT.

[*From the Pharmacological Laboratory of the Bureau of Chemistry, Washington, D. C.*]

Sodium tartrate and citrate in Locke's or in Ringer's solution or in defibrinated blood perfused through the isolated heart of the frog, dog and cat caused a decrease of cardiac activity which became more marked with increased concentrations. The action of the citrate was considerably greater than that of the tartrate, the ratio being about 5 : 1 with weak solutions. The difference was even more marked when the action of more concentrated solutions was compared, an *N*/100 citrate was as active as *N*/10 tartrate, as observed on the frog's heart. The action of citrate and oxalate was compared by its effects in calcium-free solutions (Ringer and Locke minus calcium). The effect of citrate and oxalate was found to be the same in some experiments but in others the action of the oxalate was distinctly less toxic than that that of the citrate. Since the solubility in water of calcium citrate is twice as great as calcium tartrate, while calcium oxalate is practically insoluble, it is apparent that the action of these salts is not due to the precipitation of the calcium. Again since the equimolecular solutions, calcium tartrate and calcium citrate, have the same effect as $CaCl_2$ it would also indicate that a possible decrease in ionization of the calcium in the tissues is not a factor which determines the action of tartrate, citrate and oxalate on the heart.

111 (928)

Specificity of the complement deviation test in experimental tuberculosis.

By J. BRONFENBRENNER.

[*From the Laboratories of Western Pennsylvania Hospital, Pittsburgh, Penna.*]

At the meeting of this Society on February 18, I reported the results of my preliminary attempts to prove the specificity of complement deviation in tuberculosis with Besredka's tuberculin, in some cases giving positive Wassermann reaction at the same time.

In this paper I would like to present the results of my experiment proving the possibility of independent existence of the two reactions in the same animal.

I produced an experimental orchitis in a series of rabbits, and on March 3d selected amongst them the animals giving positive W.R. All the animals were tested for Tb.R. but it was invariably negative.

When, however, infected with tuberculosis, part of these animals developed Tb.R. For a time both reactions were existing until W. R. disappeared in some animals spontaneously, in others, as shown on the table under the influence of salvarsan administered intravenously (0.04 gram per kilo), the Tb.R. persisting. The table on page 181 is the protocol of the experiment.

112 (929)

One hundred parthenogenetic generations of Daphnia without sexual forms.

By ARTHUR M. BANTA.

[*From the Station for Experimental Evolution, The Carnegie Institution of Washington.*]

November 17, 1911, the writer began rearing a number of pure lines of *Daphnia pulex* from large females taken in out-door ponds. The females were reproducing parthenogenetically and no males or "winter" eggs were found in the pond.

Rabbits No.		Rabbits with Experimental Orchitis.							Normal Rabbits (Controls).				
		100	102	105	109	111	117	118	130	131	132	133	134
March 3....	W. R.	+	+	+	+	+	+	−	−	−	−	−	−
	Tb. R.	−	−	−	−	−	−	−	−	−	−	−	−
March 5.....		Not infected			Infected with tuberculosis				Not infected		Infected with tuberculosis		
March 15...	W. R.	+	+	+	+	+	+	<<+	−	−	−	−	−
	Tb. R.	−	−	−	<+	+	<+	<<+	−	−	+	<+	<<+
March 17–21.		No salv.	Salvarsan intraven.		Salvarsan intraven.		No salvarsan		Salv. intraven.	No salv.	No salv.	Salvarsan intraven.	
March 30...	W. R.	<+	−	−	−	−	<<+	<+	−	−	−	−	−
	Tb. R.	−	−	−	+	+	<+	<+	−	−	+	+	<<+

The sign + meaning positive reaction.
The sign <+ meaning weakly positive reaction.
The sign <<+ meaning almost negative reaction.
The sign − meaning completely negative reaction.

Each line is propagated by selecting from the first brood of a young female on the day this first brood is released from the brood pouch. The selected young are placed each in an individual bottle with standard food and though examined daily are otherwise undisturbed until the first brood of the next generation appears, when the selections are made as before. Several of these lines have passed the 95th generation and one has just reached the one hundredth generation without the appearance of sexual forms in any generation. All the individuals of the first broods of each generation have been under more or less close scrutiny until they themselves reproduced. If any, or at any rate, many males had occurred they must certainly have been noticed. The method of rearing the daphnids (in individual bottles) has precluded the possibility of sexual reproduction even had males been abundant in the cultures.

There is no evidence of decreased vigor or loss of vitality in the lines. Hence it appears that there is not a necessary sexual cycle in the reproduction of this daphnid. Male daphnids, apparently of this species, have been collected at Cold Spring Harbor since this work was begun. These facts would lend evidence (if additional evidence were necessary) that the sexual cycle in *Daphnia pulex* is not an inherent necessary thing but that it is determined by environment.

Simocephalus, presumably *Simocephalus vetulus*, has been reared for 76 generations in one line, likewise without the appearance of sexual forms.

113 (930)

The comparative importance of pressure and of toxicity of trikresol in subdural injections of sera.

By C. B. FITZPATRICK, J. P. ATKINSON, and A. ZINGHER.

[*From the Department of Health Laboratories, New York City.*]

As a result of several accidents reported as being due to intraspinal administration of antimeningococcus serum containing trikresol, a number of tests were made on dogs to determine if possible whether the fatal results were due to the influence of trikresol in the serum, to the serum per se, or whether they were due to pressure.

The records were taken with the kymograph, the pressure being taken at the carotid artery. The injections were made into the vertebral artery, the femoral artery and vein, the carotid artery and into the spinal canal.

The results which are somewhat contradictory, were as follows:

An antimeningococcus serum which had produced rashes and other disturbances in patients, caused well-marked depressions in 6.5 c.c. doses. Preservatives were chloroform and 0.4 per cent trikresol. The injections were made into the femoral vein. A whole antipneumococcus serum preserved with chloroform tested in the same way produced death.[1]

Experiments (by F. and A.) with antimeningococcus serum to which was added varying quantities of trikresol (from .1 per cent to .4 per cent) gave no deleterious results when first mixed, but after standing one week in some cases depressions were obtained.

An injection of 2 c.c. of antimeningococcus serum prepared 19 days before the experiment by the addition of .4 per cent trikresol was made into the vertebral artery. A marked depression resulted. Six c.c. of this serum also injected into the vertebral artery caused immediate clotting and were followed by a convulsion with almost complete cessation of respiration.

Further experiments carried out (by F., A. and Z.) on six dogs gave the following results:

1. As a rule, there was apparently no marked disturbance of blood pressure in normal dogs immediately after the lumbar subdural administration[2] by gravity or careful gentle pressure of moderate doses of antimeningococcus sera containing .3 per cent trikresol.

2. Similar injections were made without marked disturbance in blood pressure of physiological saline solution, "*old*" antimeningococcus serum, antimeningococcus serum containing .3 per cent and .4 per cent trikresol, plain normal horse serum, normal horse serum containing chloroform, antistreptococcus serum containing chloroform and antipneumococcus serum containing chloroform.

3. Pressure appears to be a factor of real danger.

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[1] Vide, "Some Vaso-reacting Substances in Blood Serum," PROC. OF SOC. FOR EXP. BIOLOGY AND MEDICINE, 1912, LX, pp. 49–51.

[2] Lumbar puncture with skin incision through the skin and muscles was employed. Laminectomy was not used.

114 (931)

On the amino-acid content of involuting frog-larvæ.

By MAX MORSE.

[*From the Physiological Chemical Laboratories, University of Wisconsin.*]

In order to test the hypothesis that in the metamorphosis of the larvæ of the frog autolysis is the principal factor, estimation of the amino-acid content was made by the gasometric method of Van Slyke and by the formol titration method of Sörensen. Beckmann freezing point and conductivity determinations were also made since Bayliss,[1] Wells,[2] Sjöqvist[3] and others have shown that the curve of proteolysis follows that of increase of conductivity and lowering of the freezing point.

Individual tadpoles were used at first, the tails being cut from the bodies, dried in an air-blast at 40° C., pulverized in a mortar, dissolved in water to make a one per cent solution, the estimations being made upon the coagulable protein-free filtrates from boiling with basic lead acetate. About 0.40 g. dried material could be obtained from one individual, but this amount became in the neighborhood of 0.10 g. in specimens which were absorbing their tails. The determinations mentioned above gave no characteristic differences between absorbing and non-absorbing animals.

Attention was then directed to larger amounts and accordingly the dried material from a number of larvæ in the stages preceding metamorphosis was mixed and another mass was obtained from a number of individuals undergoing involution. The former gave 3.84 g. dried material, the latter 2.50 g. Upon these amounts, determinations were made as above, but again no perceptible differences in amino-acid content was found, nor were the freezing point and conductivity determinations different in the two cases. As typical examples, in conductivity experiments, with $R = 500$, $a = 58.1$ for non-absorbing and 50.3 for absorbing; for freezing points, $D = 0.72$ for non-absorbing and 0.73 for absorbing. For formol titration with 20 cm.^{3} aliquots, non-absorbing gave 0.1

[1] *Journ. Physiol.*, Vol. 36, p. 221, 1907.
[2] *Journ. Biol. Chem.*, Vol. 3, p. 35, 1907.
[3] *Skand. Arch. f. Physiol.*, Bd. 5, p. 364, 1895.

cm.^{3}, absorbing 0.3 cm.^{3} of $n/5$ $Ba[OH]_2$, in average of several determinations. VanSlyke's gave with $T = 23°$ C. and barometer = 734 mm., non-absorbing 0.34 g. nitrogen and absorbing the same. The samples were in all cases positive to ninhydrin, showing that some alpha-amino acids were present in the filtrates, but in such small amounts that they were not recognizable by the methods used.

It may be well argued, as it was in the earlier work with amino-acids in the blood, that these compounds are so quickly removed from the sphere of action that at any one time they are present in only minute quantities. It is well known that phagocytes crowd into the tissues of the metamorphosing organs after the earlier stages of dissolution are under way and it may be through their agency that the products of proteolysis are removed. Mercier[1] has been able to trace the circulation of phagocytes throughout the metamorphosing organs and the body by causing the cells to engulf carmin granules and it may be that the end products of the action of proteolytic enzymes which we must imagine to be developed at the beginning of dissolution of the muscles, etc., are taken up and carried to the body proper by these cells. This conception, however, does not give any support to the so-called phagocytic theory of involutionary phenomena, for it is quite certain that dissolution has begun before the wandering phagocytes have entered the tissues affected.

In vitro studies of autolysis of normal and involuting larvæ are in progress.

115 (932)

A simplified method for cultivating spirochaetes on liquid media.

By J. BRONFENBRENNER.

[*From the Pathological and Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

The method I wish to describe is a modification of the original method of Noguchi[2] for cultivating spirochaetes, which I think

[1] *Arch. zool. expér. et gén.*, T. 5, Ser. IVe, 1, p. 151, 1906.

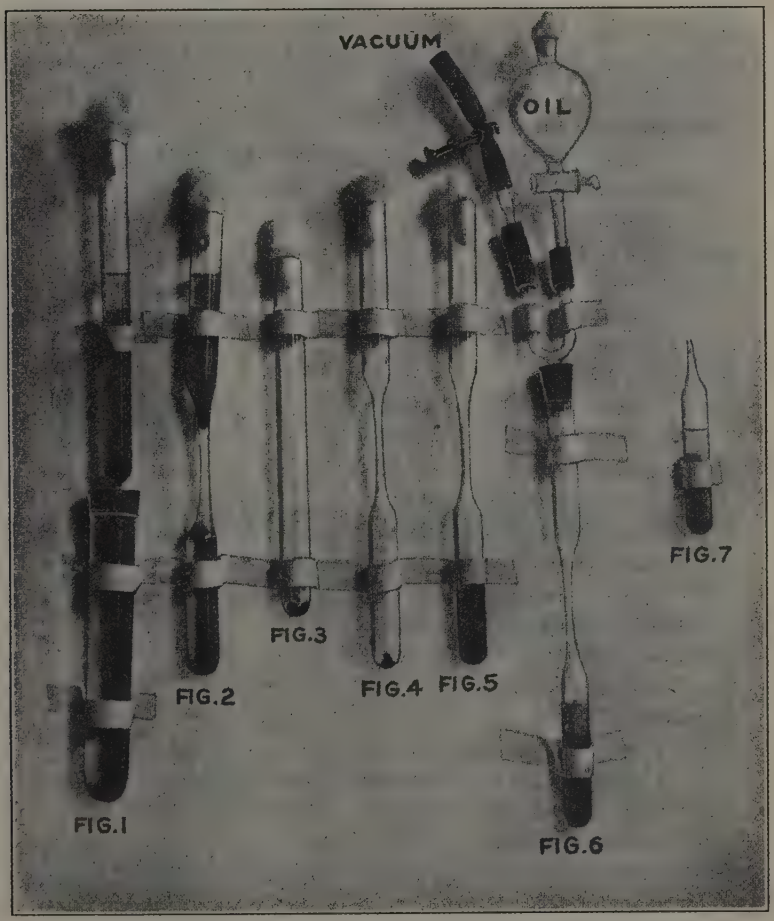
[2] *Journ. Exp. Med.*, 1912, XV, p. 211.

might be of interest to those who are called upon to cultivate these organisms. Noguchi's method, although very efficient, offers one great disadvantage, namely that the rubber stopper connection between the upper and lower parts of the apparatus often becomes a source of contamination of the culture. The method I propose consists in utilizing the principles of anaerobic cultivation in general as well as those special features which were worked out by Noguchi. This new method can be used in two different ways. Firstly, one can use the tube as shown on Fig. 2 which instead of having the rubber connection of Noguchi joining its two parts as shown in Fig. 1, is made entirely out of one glass tube; but otherwise can be used exactly as Noguchi's apparatus, namely the lower part in which the piece of rabbit kidney is put before the tube is drawn out, is filled with the ascitic broth or sheep serum water up to the point where the tube broadens out again; another piece of tissue is placed in the upper portion of the tube and this tube is filled with the ascitic agar into which the spirochaetæ culture is placed.[1] Sterile paraffine oil in a thin layer is placed above the agar and the tube is incubated. Spirochaetes during their growth filter through into the lower portion of the tube exactly as in Noguchi's method. This method is especially convenient when one intends to open the tube many times to examine its contents. The other and better way however of cultivating spirochaetes which does away entirely with the upper part of the tube,[2] is the following: I put a piece of tissue at the bottom of the tube; draw it out as before; introduce by means of a capillary pipette the spirochaetæ culture and ascitic broth in the lower tube; connect the tube with the vacuum pump, as shown in Fig. 6, warming the lower part of the tube in a water bath at 37° to facilitate the exhaustion of the air; cover the ascitic broth, after exhaustion, with sterile paraffine oil by means of a special arrangement taking advantage of the negative pressure in the tube, and finally seal the lower part of the tube at the point of strangulation

[1] It was found that spirochaetes penetrate into the lower tube more readily if 1 per cent agar is used for the ascitic-agar mixture instead of 2 per cent, as recommended by Noguchi.

[2] A Florence flask with a long neck can be used in place of the tube if a larger quantity of culture is wanted.

as shown in Fig. 7. The paraffine oil in this tube makes it possible to preserve spirochaetes alive even after the tube is once opened. By this simple method I have been carrying successfully my subcultures of spirochaetes for the last three months.



RECAPITULATION OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE COMMUNICATIONS.

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electrolytes in determining the equilibrium of aqueous oil systems.

819. On analogous effects exerted by antagonistic calcium and citrate ions in physical and biological systems.

820. [with **F. West.**] On the rôle played by antagonistic ions in the process of blood coagulation.

821. On analogous effects exerted by anesthetics in physical and biological systems.

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883. Experiments dealing with the relation of the sinus node to the effects of stimulation of the vagus nerve.

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894. [with **Zeno Osterberg.**] The pressor substances of the pituitary gland.

895. See **Will, E. Barbara.**

Crehore, A. C.

852. [with **H. B. Williams.**] Electric currents in conductors with distributed capacity considered in relation to the propagation of the nerve impulse.

Cullen, G. E.

850. See **Van Slyke, D. D.**

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831. [with **Emil Goetsch.**] Hibernation and the pituitary body.

832. See **Goetsch, E.**

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836. [with **J. Rosenbloom.**] Metabolism studies in a case of myasthenia gravis.

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859. See **Carlson, A. J.**

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837. [with **S. A. Munford.**] A case of interpolated extra systoles, illustrated by electro-cardiograms.

Dwyer, J. G.

860. See **Zinsser, H.**

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924. The synthesis and rate of elimination of hippuric acid after benzoate ingestion in man.

Livingston, A. E.

856. Effect of castration on the weight of the pituitary in rabbits.

857. Effect of thyroidectomy, followed by thyroid feeding, on weight of the pituitary in rabbits.

Loeb, L.

838. See **Lathrop, A. E. C.**

839. See **Lathrop, A. E. C.**

881. The experimental production of an early stage of extrauterine pregnancy.

Lusk, Graham.

845. The influence of epinephrin on carbohydrate metabolism.

920. The specific dynamic action of levulose, glycocoll and alanin in phlorhizin glycosuria.

Marine, David.

891. Parathyroid hypertrophy and hyperplasia in fowls.

MacCallum, W. G.

863. [with **R. A. Lambert.**] Modifications of the Abel vividiffusion apparatus, with demonstration.

MacNeal, W. G.

869. See **Siler, J. F.**

870. See **Siler, J. F.**

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916. On the difference in the response of animals of different ages to a constant quantity of uranium nitrate.

McCollum, E. V.

878. [with **Marguerite Davis.**] Further observations on the physiological properties of the lipins of the egg-yolk.

McCoy, G. W.

842. See **Ophuls, W.**

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904. [with **J. Rosenbloom.**] Metabolism studies in the case of congenital hemolytic jaundice with splenomegaly.

882. The influence of the diaphragm descent on the movements of the heart.

Will, E. Barbara.

895. [with **A. C. Crawford.**] Note on the action of epinephrin on the guinea-pig uterus.

Williams, H. B.

852. See **Crehore, A. C.**

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828. [with **J. F. Lyman.**] Creatine in the muscle tissue of the lamprey.

Wilson, J. Gordon.

896. [with **F. H. Pike.**] A comparison of the effects of labyrinthine and cerebellar lesions in the turtle.

Wollstein, Martha.

874. [with **S. J. Meltzer.**] The pulmonary reaction to *B. pyocyaneus*.

Wood, F. C.

902. [with **F. Prime.**] The action of radium on growing cells.

Woodruff, L. L.

855. Further light on the conjugation of *Paramecium*.

860. [with **Rh. Erdmann.**] Complete periodic nuclear reorganization without cell fusion in a pedigreed race of *Paramecium*.

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915. See **Sansum, W. D.**

VanSlyke, D. D.

830. [with **G. M. Meyer.**] The effect of protein starvation and feeding on the amino-acid content of the tissues.

850. [with **G. E. Cullen.**] Preparation of soy bean urease in solid form and its use in urea determination.

Zingher, A.

930. See **Fitzpatrick, C. B.**

Zinsser, Hans.

861. [with **J. G. Dwyer.**] On the aggressin-like action of anaphylatoxin.

Zucker, T. F.

853. Blood platelets and blood clotting.

EXECUTIVE PROCEEDINGS.

Fifty-fifth Meeting.

Cornell University Medical College, October 15, 1913. President Ewing in the chair.

Members present: Adler, Atkinson, Beebe, Butterfield, Calkins, Clowes, Cohn, DuBois, Eggleston, Emerson, Ewing, J., Field, Fitzpatrick, Goldfarb, Hartwell, Hatcher, Jackson, Kleiner, Lambert, Lusk, Meltzer, Mendel, Murlin, Myers, Norris, Ringer, Rous, VanSlyke, Wallace, White, Wiggers, Winslow.

Fourth Meeting.

Pacific Coast Branch.

San Francisco, California, October 9, 1913.

Members present: Cooke, Gay, Manwaring, Ophuls.

Fifty-sixth Meeting.

College of Physicians and Surgeons, December 17, 1913. President Ewing in the chair.

Members present: Atkinson, Auer, Benedict, S. R., Brooks, Burton-Opitz, Eggleston, Eisenbrey, Emerson, Ewing, J., Field, Githens, Hartwell, Harris, Jackson, Kleiner, Lambert, Lee, Levin, Longcope, Meltzer, Meyer, G., Murlin, Myers, McClendon, Norris, Oppenheimer, Pike, Scott, E. L., Terry, VanSlyke, Wadsworth, West, Wiggers, Winslow.

Members elected: J. A. E. Eyster, M. S. Fine.

Resolutions adopted by the Council: Each member of the Society shall be allowed each year, free of charge, two printed pages in the Proceedings; space in excess of this will be charged, at cost, at the rate of $2.25 per page.

Fifty-seventh Meeting (Eleventh Annual Meeting).

The Rockefeller Institute for Medical Research, February 18, 1914. President Ewing in the chair.

Members present: Atkinson, Auer, Bronfenbrenner, Butterfield, Cohn, Cole, R. J., Ewing, E. M., Ewing, J., Falk, Fine, Flexner, Gies, Githens, Harris, Hess, Jackson, Kast, Lambert, Longcope, Lusk, MacCallum, MacNeal, Meltzer, Murlin, Myers, Norris, Pappenheimer, Simpson, Swift, Terry, Wallace, Weil.

Members elected: R. E. Swain, Henry Laurens, K. F. Meyer, W. H. Brown, C. G. Bull, H. C. Bailey, Reinhard Beutner, George Pierce.

Officers elected: President, Graham Lusk; Vice-President, W. J. Gies; Treasurer, J. R. Murlin; Secretary, Holmes C. Jackson.

Resignation of J. F. McClendon read and accepted.

Fifth Meeting.

Pacific Coast Branch.

San Francisco, California, April 9, 1914.

Members present: Crawford, Cooke, Meyer, K. F.

Fifty-eighth Meeting.

University and Bellevue Hospital Medical College, April 15, 1914. Vice-President Gies in the chair.

Members present: Beutner, DuBois, Ewing, E. M., Fine, Gies, Harris, Howe, Jackson, Lambert, Mandel, J. A., Meltzer, Murlin, Myers, Senior, VanSlyke, Wallace, Wiggins.

Members elected: J. F. Siler, F. F. Russell, H. N. Evans, W. P. Lucas, C. P. Hodge.

Fifty-ninth Meeting.

Schermerhorn Hall, Columbia University, May 20, 1914. President Lusk in the chair.

Members present: Atkinson, Beebe, Benedict, Calkins, Eggleston, Eisenbrey, Field, Fine, Gies, Githens, Goldfarb, Harris, Hess,

Howe, Jackson, Lambert, Lee, Levene, Lusk, Meltzer, Meyers, G. M., Morgan, Mosenthal, Murlin, Myers, Pappenheimer, Ringer, Stockard, Swift, Weil, Wilson, Wood.

Members elected: I. Greenwald.

The President appointed an editorial committeee to supervise the preparation of the program and the selection of members to discuss the various papers presented before the Society.

The resignation of Ernst Sachs and F. W. Bancroft read and accepted.

Sixtieth Meeting (Special).

Carnegie Laboratory for Experimental Evolution, Cold Spring Harbor, June 6, 1914. President Lusk in the chair.

Members present: Banta, Beebe, Berg, Beutner, Cohn, Davenport, DuBois, Eggleston, Ewing, J., Fitzpatrick, Gies, Githens, Goldfarb, Gortner, Jackson, Lee, Lusk, MacCallum, Mendel, Meltzer, Morgan, Murlin, Pike, Ringer, Scott, E. L., Senior, Torrey, Wallace, Weil, Wiggers, Williams, H. B.

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ADLER, HERMAN M.....Psychopathic Hospital, 74 Fenwood Road, Boston.
ADLER, ISAAC.....New York Polyclinic Medical School.
ALSBERG, CARL.....U. S. Department of Agriculture, Washington, D. C.
ANDERSON, JOHN F.....U. S. Public Health and Marine-Hospital Service. Hygienic Laboratory, Washington, D. C.
ATKINSON, JAMES P.....Department of Health, New York City.
AUER, JOHN.....Rockefeller Institute for Medical Research.
AUSTIN, J. H.....University of Pennsylvania.

BAILEY, H. C.....Cornell University Medical College.
BANTA, A. M.....Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, Long Island, N. Y.
BANZHAF, EDWIN J.....Department of Health, New York City.
BARDEEN, CHARLES R.....University of Wisconsin.
BEEBE, SILAS P.....Cornell University Medical College.
BENEDICT, FRANCIS G.....Nutrition Laboratory, Carnegie Institution, Boston.
BENEDICT, STANLEY R.....Cornell University Medical College.
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BERGEY, DAVID H.....University of Pennsylvania.
BEUTNER, REINHARD.....Rockefeller Institute for Medical Research.
BIRCHARD, F. J.....Dominion Laboratory, Winnipeg, Man., Canada.
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